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REFLEXIVE CATALYSIS, A POSSIBLE MECHANISM OF MOLECULAR DUPLICATION IN PREBIOLOGICAL EVOLUTION

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Oparin (1938) described the origin of life in terms of simple colloidal droplets of proteins or polypeptides that acquired increasing complexity under the influence of natural selection. This account dealt inadequately with two problems. First, spontaneous peptide formation in the ocean is thermodynamically improbable (Borsook, 1950). Solutions to this problem have been offered in several forms. Blum (1955) has suggested that an energy-transfer mechanism involving ATP first appeared spontaneously, and made possible the subsequent multiplication of proteins and polynucleotides. Lanham (1952) suggested that amino acids were originally synthesized in some high-energy form that resulted in their ready polymerization. Wald (1954) meets the same problem by supposing that the crystal-like properties seen in aggregates of many organic compounds stabilized growing polypeptides and so tipped the balance in favor of their synthesis. Perhaps the most promising approach has been the assumption that polypeptides were pre-formed under anhydrous conditions (Fox, 1955) or in shallow pools concentrated by evaporation (Blum, 1955).

The second weakness in Oparin's theory is the unsupported assumption of effective natural selection during chemical evolution. His "coacervate droplets" have no stable mechanism for transmitting their individual characteristics, but are determined by their environment. Variant lines favored by selection would inevitably revert to the original type within a few generations. Biologists generally agree that natural selection can operate only among self-replicating, mutable units (Plunkett, 1944; Muller, 1947; Beadle, 1949; Blum, 1955; Dobzhansky, 1955). Since the simplest known structures exhibiting this behavior are nucleoproteins, it is also generally agreed that, prior to spontaneous formation of the first nucleoprotein gene or virus, chemical evolution had to proceed without the directing and conserving influence of natural selection.

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The initiation of chemical evolution by inorganic synthesis of simple organic molecules has been simulated in a variety of experiments (Garrison, *et al.*, 1951; Bahadur, 1954; Varner and Burrell, 1955; Miller, 1955; Fox, 1955). Processes by which these building blocks might have been organized into genes or protoplasm are as yet almost entirely hypothetical. Without natural selection, reactions must have been random, at least in the sense that molecules were synthesized according to their relative facility of spontaneous occurrence *without any relation to their potential biological usefulness.*

The purpose of this paper is to show that Oparin's implication of natural selection prior to the origin of genes can be reconciled with genetic theory, and that there probably are self-duplicating, mutable systems of simpler chemical constitution than nucleoproteins. Such units would, by their nature, propagate themselves differentially according to their relative reproductive rates, that is according to their biological efficiency. If natural selection in this form did in fact operate at an early stage in chemical evolution, then the emergence of organized protoplasm, energy transfer systems and nucleoproteins would not be inorganic phenomena, but would have the same probability as other difficult inventions of organic evolution.

Given enough time and enough building blocks, there is little doubt that random reactions might produce nucleoproteins, but it is probable that such catalytically active compounds as pyridoxal and heme (Metzler, *et al.*, 1954; Grannick, 1953; Langenbeck, 1953) were produced in much larger quantity and perhaps much earlier (Horowitz, 1945). The possible role of such catalysts in chemical evolution has received little attention. Apparently no attention has been given to the fact that, theoretically, a simple organic catalyst can initiate a self-duplicating system analogous to a gene.[2] This is the apparent effect of catalysis of an early step in a reaction sequence that leads to synthesis of the catalyst, and the mechanism suggests the term *reflexive catalysis.*

CYCLES OF MOLECULAR REPRODUCTION

The older concept of the gene as a single autocatalyzing molecule (Troland, 1914, 1917) has lost some of its force in the light of newer concepts of nucleic acid structure, especially the proposal of Watson and Crick (1953). If, in fact, each gene (or chromosome) consists of two complementary strands or templates, only one of which is regenerated in the formation of each daughter gene, duplication may be regarded as a two-step cycle (cf. Friedrich-Freksa, 1940), completed in a minimum of two successive cell generations. For if a mutation occurs in one of the two filaments in a chromosome, only one of the daughter cells will receive this filament, while the other daughter cell will regenerate a complete, unmutated chromo-

[2] The author is grateful to Dr. George Wald and Dr. Werner Maas for encouragement and criticisms during the development of this idea.

Since this paper was submitted to the editor, the same principle has been suggested in an article by M. Calvin (1956).

some. A second filament exactly like the original mutant will appear only at the next cell division, when two of the four granddaughter chromosomes will be mutant. Such step-wise synthesis of the gene suggests the possible extension of the gene concept to a three- or four- or multistep cycle. To be sure, the intercalation of extra steps does not at once appear to simplify the gene concept, but its consequences will be discussed below.

In the remainder of this article the term *gene* will apply to a nucleoprotein molecule, or any segment of such a molecule, which serves as a template for its duplication in either a one or a two-step cycle. The term *gene-analog* will refer to any cycle of compounds simpler than nucleoproteins whose components will be generated serially after an appropriate environment is primed with a component of the cycle.

Both genes and gene-analogs must, by hypothesis, be able to transmit to one or more environments the information required for their approximate replication. To distinguish it from the next phenomenon, this process may be called the *cycle of self-duplication,* although self-duplication on a molecular level can hardly be analogous to self-duplication by cells (Claude, 1949). Most modern genes also transmit information to their environment for the synthesis of some other molecule. This is useful to the organism and thereby helps to insure long-term perpetuation of the chemical environment upon which the genes depend. The transmission of this second type of information, and its consequences in reproduction of the gene, may be called the *cycle of the organism.*

The combination of these two types of cycle evidently had a tremendous advantage very early in the history of evolution by coordinating immediate duplication of the gene with perpetuation of its supporting protoplasm. Nevertheless, careful consideration will reveal that, theoretically, either kind of cycle can by itself achieve specific molecular reproduction, which is the essence of heredity (Wilson, 1925).

That the cycle of the organism can be dispensed with is illustrated by viruses insofar as these reproduce at the expense of the host organism. It is more difficult to see how heredity can operate without a cycle of self-duplication, without a gene-analog. An organism surely could not faithfully reproduce so complex and variable a molecule as a nucleic acid without a model to work from. But no model is necessary for the production of relatively simple molecules. The adult types of porphyrin are probably not all represented in the zygote. Certainly a compound like pyridoxal phosphate might be produced slowly but unerringly in a system that contained neither pyridoxal nor protein. Hence, in a hypothetical cell with only simple constituents (including catalysts), all components might increase without direct participation of any molecule in its own synthesis. In general, any molecule that is regularly synthesized by a chemical system and that aids in the maintenance of that system is effecting its own propagation in the same sense as one of the paired filaments of the Watson-Crick model.

A hypothetical cell like that mentioned above might, then, contain no self-duplicating cycles. Yet if it contained many molecules of each essen-

tial catalyst, it could theoretically reproduce each catalyst several fold in the course of one growth cycle. When such a cell fragmented there would be a considerable possibility that at least one of the daughter cells would receive compatible quantities of all catalysts. Despite a very high reproductive mortality, with extinction of many lines, such a cell might propagate its kind successfully for countless generations, or until a more efficient competing or predator species appeared.

This model, though embodying a system of heredity not specified for Oparin's coacervate droplets, is hardly more responsive to natural selection. It would probably not arise in nature, and if it did it would lead to nothing higher by direct protoplasmic continuity. For although self-determining it would be virtually incapable of mutation. Daughter cells that differed significantly from the parent type might all be inviable, and if one survived and produced a useful new catalyst, its descendants would almost certainly revert to the ancestral metabolic pattern. Evolution of such cells, if it occurred at all, would be by saltation, that is, essentially by *de novo* origin of new types of cell to compete with the old. At the level of complexity and stability seemingly required, such spontaneous generation would offer little help in explaining the origin of life.

REFLEXIVE CATALYSIS

Simplified to the logical extreme, the "cell" described above would contain a single catalyst, maintaining one critical reaction or group of reactions. From the products of this catalysis, together with substances received from the environment, all other constituents would have to be derived in spontaneous reactions, including the catalyst itself.

Such a system is highly unlikely in droplet form, but as a theoretical construct it is of key importance. For at this point the cycle of the organism becomes identical with the cycle of self-duplication. The catalyst in this system conveys a *single* item of information which is both necessary for maintenance of the system as a whole and sufficient by itself for duplication of the catalyst when applied repeatedly in the synthesis of many molecules of a precursor. In contrast, a gene that participates in both kinds of cycle conveys *two* items of information, though it may conceivably transmit them simultaneously, and the information for self-duplication need be applied only once.

Perhaps more important is the fact that, if none of the reactions depended on spatial proximity of reagents as would be the case with unstable combining moieties, such a one-catalyst system could maintain itself outside an "organism," in solution. A gene-analog of this type would be constituted by any chemical system that occasionally synthesized its own key catalyst when provided with appropriate starting materials. It appears that such a system does not violate any physical laws or even any common conceptions of chemistry. In fact, if one accepts a very low level of efficiency and a very slow rate of reproduction, it is likely that many organic compounds would be found to qualify, under peculiar conditions, as key

catalysts in self-duplicating cycles. Any compound that thus catalyzes the synthesis of one of its precursors may be called a *reflexive catalyst.*

The following reaction scheme may be taken as a general definition of reflexive catalysis:

$$A \xrightarrow{(X_n)} X_1 \longrightarrow (\cdots\cdot) \longrightarrow X_n$$

However inefficient any such system may be, it shares one essential characteristic of the gene: X_n accumulates at a rate that increases, relative to concentration of simple precursors (A), when the concentration of X_n increases.

If reflexive catalytic cycles are to furnish a basis for evolution analogous to that provided by genes, mutability is essential. However, genetic material may make errors in duplication that are not mutations in the sense of being transmissible. Some variant viruses may reproduce the parental type instead of their own type (Wang and Commoner, 1954), and some may not reproduce at all. Among reflexive catalytic cycles this behavior would surely predominate over true mutation. Isomers of the essential compounds produced as by-products might contribute to the parent cycle, but more often they would be inactive. On rare occasions, but perhaps less rare than the mutation of modern genes, such a by-product might initiate an altogether new cycle of which it was a regular product. It would then enter into competition or cooperation with the established cycles at a rate determined by its relative efficiency.

When brought down to the level of simple molecules, the discontinuities of organic evolution can be nothing less than saltations (Troland, 1914). A new cycle would bear little or no relation to that from which it took its origin, and might in fact combine products of several established cycles.

Selection would probably result in some forms of cooperation or "symbiosis" (Wright, 1949). For example, two compounds might achieve reflexive catalysis each by facilitating the synthesis of a precursor of the other. Such a gene-analog involving two catalysts would be analogous to the Watson-Crick model of two complementary templates.

MATHEMATICAL DEMONSTRATION

The self-duplicating chemical systems postulated in the preceding section are supposed to propagate themselves without the assistance of protoplasm among the compounds dissolved in a primordial ocean. That such propagation must be subject to natural selection follows from the theoretical discussions of self-duplication already cited. However, the radical differences between these gene-analogs and conventional genes makes a mathematical demonstration desirable. In a model, the first reflexive catalytic cycle should multiply exponentially; it should give rise to a variety of new cycles, and these should compete in such a way that the more efficient "mutants" will eventually predominate. Multiplication of a reflexive catalyst would not follow a simple exponential curve because

spontaneous generation, negligible in the case of genes or organisms, is here an appreciable quantity. Thus in figures 1B and 1C the slopes of some curves increase as catalytic synthesis assumes the dominant role.

Table 1 defines two hypothetical models of chemical evolution. The first two columns outline a sequence of synthetic processes of the type usually postulated for the early stages of chemical evolution, beginning with simple organic precursors, X and Y. In the last two columns are

TABLE 1

HYPOTHETICAL SCHEMES OF CHEMICAL EVOLUTION

Steps	Reactions	Rate	
		Model I	Model II
1	$2X \rightarrow Z$	1000	1000
2	$2Z \rightarrow U$ $2Z \rightarrow V$ $2Z \rightarrow W$	$\frac{3z^2}{10^8}$	$\frac{3z^2}{10^8}$
3	$2Z + Y \rightarrow A$ $2Z + Y \rightarrow B$ $2Z + Y \rightarrow C$	$\frac{z^2}{10^8}$	$\frac{z^2}{10^8}(1+f)$
4	$A + B \rightarrow D$ $A + B \rightarrow E$	$\frac{ab}{10^6}\left(1+\frac{u}{10}\right)$	$\frac{ab}{10^6}\left(1+\frac{u}{10}\right)$
5	$D + W \rightarrow F$	$\frac{dw}{10^8}$	$\frac{dw}{10^8}$
6a	$C + E \rightarrow G$ $C + E \rightarrow H$	$\frac{ce}{10^8}$	$\frac{ce}{10^8}(1+k)$
6b	$C + E \rightarrow I$ $C + E \rightarrow J$		$\frac{ce}{10^8}(1+k+l)$
7	$G + D \rightarrow K$	$\frac{gd}{10^6}$	$\frac{gd}{10^6}$
8	$I + D \rightarrow L$	$\frac{id}{10^7}$	$\frac{id}{10^7}$

reaction rates arbitrarily assigned so as to illustrate the difference in chemical evolution without and with reflexive catalysis, Models I and II, respectively. Small letters in the expressions for reaction rates stand for relative concentration; they might, for example, represent moles per cubic mile of ocean. The unit of time might be a decade or a century. Each rate applies to all reactions listed under the same step.

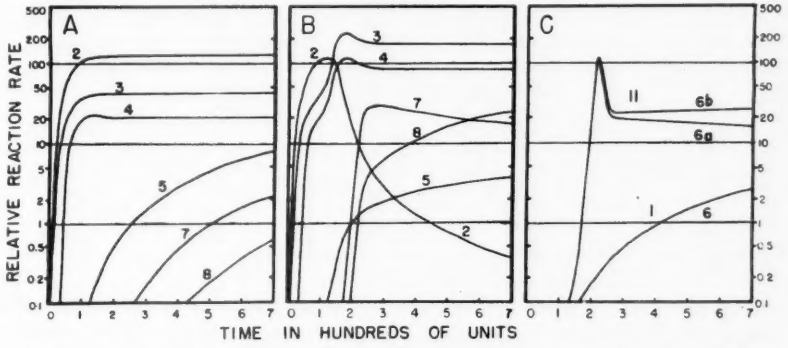

FIGURE 1. Rates of synthesis in chemical evolution. A. Without reflexive catalysis (Model I). B. With reflexive catalysis (Model II). C. Step 6 contrasted for the two models and showing the relative acceleration and the differentiation made possible by reflexive catalysis. (Step 1 is constant at 1000.)

Figure 1 illustrates the changing reaction rates plotted on a semi-logarithmic scale. The rates are functions of the concentrations of the reagents, which change with time. Calculations were based on expressions for net rate of increase of each compound, synthesis minus utilization. Thus, in Model I, Z accumulates at the rate,

$$1000 - \frac{24z^2}{10^8},$$

while in Model II the rate is

$$1000 - \frac{18z^2}{10^8} - \frac{6z^2}{10^8}(1 + f)$$

Cumulative concentrations were calculated approximately for regular time intervals appropriate to the rate of change. Obtained concentrations for times 300 and 700 appear in table 2, where whole numbers have been retained without rounding in order to clarify some of the relations among the variables (e.g., $w = u - f$).

Under the assumptions of the models, X and Y are available from geological sources in unlimited quantity. Under the activating influence of heat, electricity, or radiation, X is converted to Z at a rate which remains nearly constant throughout the time interval concerned. Further reactions occur

TABLE 2

CONCENTRATION OF PRODUCTS IN MOLES PER UNIT VOLUME OF OCEAN

Compound	At time 300		At time 700	
	Model I	Model II	Model I	Model II
Z	64,600	9,760	64,600	3,738
U,V	29,432	12,753	79,432	13,125
W	29,315	12,563	77,432	11,759
A,B	85	260	26	251
C	9,669	17,880	23,951	52,183
D	5,113	14,970	11,062	32,160
E	5,080	48	11,023	3
F	117	190	2,000	1,366
G	29	1,913	203	530
H	38	4,084	637	11,523
I	37	4,237	545	7,752
J	38	4,584	637	14,949
K	9	2,171	434	10,993
L	1	347	92	7,197

spontaneously at different rates, and are treated here as irreversible. The products are assumed to be stable except as they participate in other indicated reactions. These simplifying assumptions are not essential, but appear to represent conditions that would be approximately fulfilled in many natural systems. Each step is presumed to be nonspecific, but for simplicity only a small number of alternate reactions is shown in each case and these are treated as if equal in rate. Compensating degradation products, understood to occur in most steps, are omitted, as are countless other reactions that would probably occur without contributing to evolution. The effect of all the by-products thus omitted from the scheme would be to reduce the effective utilization of the starting materials without altering the picture qualitatively. As will soon become apparent, side reactions at the primitive levels would gradually be shut off as higher systems of reflexive catalysis evolved, so that their omission minimizes the difference between Models I and II.

For uncatalyzed reactions, or for reactions catalyzed by physical agents, the rate given is directly proportional to the concentration of the reagents. Concentration of the variable reagents is represented in the numerator of the rate expressions; z^2, ab, etc. The remainder of each rate ($3/10^8$, etc.) is an arbitrarily chosen constant. Catalysis by a compound that varies with time is indicated in a second term consisting of the basic rate multiplied by the concentration of the catalyst and by a second constant. Thus, unit concentration of U increases the rate of step 4 by a factor of 1.1 over the uncatalyzed reactions. When u reaches ten units, the rate of step 4 becomes twice as great as when u was zero. In Model II, steps 3 and 6 are modified by reflexive catalysis. The rates of these reactions increase, like that of step 4, as their respective catalysts accumulate, but unlike step 4 these steps lead indirectly to synthesis of their own catalysts.

Figure 1A represents Model I except for step 6, which appears separately in figure 1C. This figure therefore portrays chemical evolution without any gene-analogs. As long as geological conditions remain the same, the rates of the several reactions retain their relative positions. No reaction, once established, is slowed or extinguished by competition with reactions that begin later. Figure 1B shows the same reactions modified by reflexive catalysis at only two of the eight steps. The striking difference between this and figure 1A is that here the curves cross one another as later reactions outstrip earlier ones.

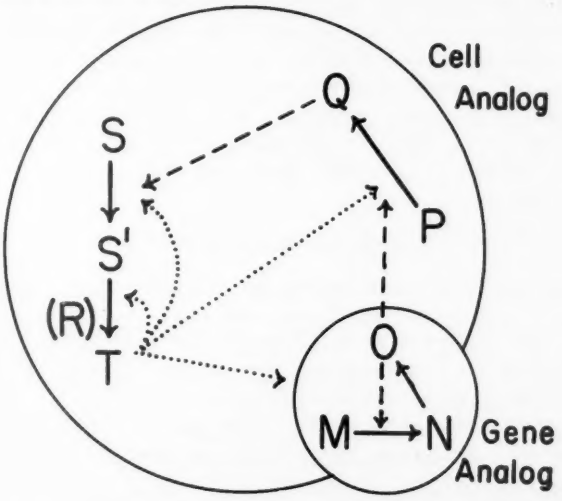

FIGURE 2. The primordial cell. ("⟶" signifies chemical transformation; "- - -→", catalysis; "·····→", indirect facilitation.) T facilitates each catalyzed reaction by forming a membrane that tends to retain catalysts. By catalyzing the reaction M⟶N, O completes the "cycle of self-duplication". By retaining O for this catalysis, T completes the "cycle of the organism."

Of central importance in both models is a competition between steps 2 and 3 for the common precursor, Z. It is evident from table 1 and from the figure that initially step 2 has a three-fold advantage over step 3. In Model I this advantage is maintained indefinitely. In Model II, however, step 3 rapidly picks up speed after the catalyst, F, begins to appear in reaction 5. The catalyst for step 3 accumulates at a rate which increases with the rate of step 3, so that both rates tend to rise exponentially until the accumulated excess of starting material, Z, is exhausted. The eventually high concentration of F diverts Z to step 3 as fast as it becomes available, and thus virtually extinguishes step 2.

Steps 7 and 8 in Model II illustrate competition between two reflexive catalysts, K and L, for the common precursor, D. In Model I, step 7 proceeds nearly ten times as fast as step 8 and maintains this advantage indefinitely. In Model II the products of both reactions catalyze step 6 and form competing cycles. Since L catalyzes its precursor more specifically,

it eventually dominates step 6 and reverses the original difference in rates between steps 7 and 8.

Table 2 compares the quantities of compounds accumulated in Model I and Model II. At time 300 the most noticeable difference is the greater abundance in Model II of the compounds having the most complex derivations, G through L. At time 700, Model II has added very little of the inert compounds, U and V, which do not form further combinations in the scheme. In contrast, some of the more reactive compounds are being produced in abundance and consumed with increasing efficiency in the production of the most effective catalysts. It appears from this model that even at a very early stage in chemical evolution, as in the modern biological world, the simplest organic compounds would have been rapidly consumed in the synthesis of the currently most complex molecules and in the compensating degradations. The complex molecules thus produced in variety and abundance by reflexive catalysis would make more probable the early origin of useful new catalysts and would shorten the time required for emergence of the first nucleoproteins.

BEGINNINGS OF PROTOPLASM

The aggregation of organic matter into the primordial cells or droplets was discussed at length by Oparin, and relevant advances in chemical knowledge were reviewed by Wald (1954). The genetic problems have not been solved, and it is not apparent that natural selection can operate, as Oparin supposed, among droplets having no fixed heredity. Moreover, in the earliest evolution of protoplasm there would be frequent interruptions in the continuity of descent among cells, and such continuity is essential for their natural selection. Even given continuity of descent, natural selection among droplets would still operate inefficiently if key compounds were too readily diffusable from one lineage to another. In this circumstance natural selection would exercise control not at the cellular, but at the molecular level. With this modification, Oparin's assumption that natural selection assisted in organizing the first protoplasm or "eoplasm" (Troland, 1917) can be defended.

Reflexive catalysis need not, in principle, involve direct chemical interaction between "catalyst" and starting compounds. Any chemical or physical influence exerted by the key compound to facilitate the initial step of the cycle should favor eventual formation of the catalyst. The necessary effect would be achieved, for example, if the "catalyst" merely helped to stabilize colloid particles or droplets in which it or a precursor was synthesized. Thus the distinction between the cycle of self-duplication and the cycle of the organism is bridged, and on this may depend the otherwise difficult transition from genes or gene-analogs multiplying independently in solution to genes or gene-analogs participating in protoplasmic functions.

Many compounds, like polypeptides, would probably be synthesized more readily in semi-organized droplets than in solution. Let us suppose, then,

that compound T stabilized colloidal droplets by arranging itself as a membrane on the surface; that the precursor for T, S, is abundantly available, but that synthesis of T at a significant rate depends upon the presence in one droplet of two catalysts, Q and R (or R could equally well be a reagent combining with S′):

$$S \xleftarrow{(Q)} (S') \xrightarrow[\longleftarrow]{(R)} T$$

Under the assumptions of this model, when the original droplet was disrupted, net synthesis of T would be likely to cease. But if Q and R were regular, albeit rare products of common reactions, they would occasionally be produced together in other droplets. Such droplets, by virtue of their tendency to acquire a "membrane," would be relatively long-lived and would have a better opportunity to synthesize Q and R than other droplets would have to synthesize their respective, useless compounds. Thus, in many life-cycles of transient droplets, Q and R would synthesize large quantities of T, and T would itself insure repeated synthesis of Q and R. If any of the compounds were stable enough to accumulate, this would accelerate synthesis of all three.

This sort of system, even if it never evolved to a higher level of efficiency, would contribute valuable compounds to chemical evolution and would propagate itself on a molecular level in the absence of more efficient competing cycles. After prolonged accumulation in the ocean, compound T might finally become common property of all droplets, making them more stable sites for further organic syntheses.

As long as T remained rare, such a cycle might contain the potentiality for further evolution. Just as Q and R would be favored by the stabilization of droplets in which they were synthesized, more remote precursors or catalysts, exemplified in figure 2 by P, would be favored to a lower degree. Thus a "channel" would be made for all reactions contributing to the synthesis of T. Now if any of the compounds, P, Q, or R, should be a member or a by-product of a reflexive catalytic cycle, such as M-N-O in the figure, a link would be established between the cycle of the organism involving T and the cycle of self-duplication. In the figure, M-N-O is a self-duplicating cycle that operates more efficiently in the droplet than free in solution because of the presumed higher concentration of O inside the membrane. The conversion of P to Q can be regarded as a side reaction of this catalysis, and T is then a remote by-product. Like a useful gene, the M-N-O cycle would be favored by natural selection, and the quantity of N and O in the ocean would increase as a result of repeated syntheses in the relatively long-lived droplets which they helped to build.

(Although the retrograde extension of selection here described is not necessary, it might be usual in the early stages of evolution. The gene-analog would not be strongly favored through its useful remote product until the first type of selection, or "channeling," had produced some abun-

dance of other necessary reagents and catalysts. Only then would the gene-analog frequently achieve "phenotypic" expression.)

EXPERIMENTAL IMPLICATIONS

Experiments have shown conclusively that amino acids and similar simple molecules could have been produced in abundance under possible primitive earth conditions. There is some evidence, too, that similar reactions might have yielded polymers (Fox, 1955). Attempts to extend this evidence to proteins, nucleic acids and nucleoproteins are based on the assumption that these syntheses, also, must at first have been essentially inorganic. The possibility of self-propagating systems and natural selection among compounds simpler than proteins opens another experimental approach to the origin of life. If reflexive catalysis operated, as seems possible, in dilute solutions, the chemical environment would have acquired combinations of catalysts that carried out syntheses cooperatively. By the time any compounds attained such complexity as that of nucleoproteins, colloidal droplets may have been prevalent and syntheses may have been qualitatively very much like those of modern protoplasm.

The production of life-like chemical systems may, in fact, be easier than the inorganic synthesis of life-like chemical compounds. From this point of view it would seem profitable to direct attempts toward the construction of simple self-duplicating systems capable of evolving. The eventual chemical products of such a system would be as unpredictable as those of biological evolution itself. As always with models of evolution, however, the time factor would have to be dealt with. Prebiological evolution among reflexive catalysts would surely have been a slow process, and if reproduced in the laboratory it might not even be measurable. However, as with Drosophila evolution (Wallace, 1951), there may be ways of speeding up a laboratory model without destroying its essential analogy to the natural phenomenon. (1) Concentrations in the laboratory could probably be a thousand or a million times as great as in the prebiological ocean. (2) Temperature of the solution, if maintained near 100°C, would probably be significantly higher than during most of chemical evolution. (3) Some of the key catalysts might be supplied or supplemented by the experimenter to obviate the long interval that usually would have preceded their chance origin in prebiological evolution as well as the interval otherwise required for their initial slow multiplication. (4) If life originated in the open ocean rather than in lakes, Nature undertook only one experiment. In the laboratory, several or many experiments could be conducted simultaneously. Fruitful experiments might be conducted in heated, constant-volume tanks. A mixture of selected compounds fed slowly into such a tank would undergo some spontaneous chemical changes: degradations, molecular rearrangements, and syntheses. In most such experiments, and according to conventional views in all, the changes observed in the outflow liquor should commence at once to approach a steady state in asymptotic fashion. In a mixture one of whose components or products had reflexive catalytic

activity, however, the initial approach to a steady state would not be asymptotic for all compounds. Over a longer period, perhaps several years, the compounds constituting self-duplicating cycles would tend to increase geometrically. This would demonstrate reflexive catalysis. If a new compound later appeared and showed similar geometric increase, it would illustrate the capacity of the system to evolve toward higher complexity.

SUMMARY

Oparin's account of the origin of life assumed the possibility of natural selection among unorganized colloid droplets. This is not consistent with modern views of natural selection, which would require self-duplicating, mutable systems. On the other hand, the common conception of of nucleoprotein genes as the simplest self-duplicating mutable units may be mistaken. Any molecule that catalyzed the synthesis of one of its precursors, in a system where these precursors eventually formed more of the same molecule, would be reproducing itself in the sense required by natural selection. The term reflexive catalysis is suggested for this process to distinguish it from autocatalysis.

Molecular reproduction by reflexive catalysis would generally have a low specificity or "high mutability." It might at any time result in new molecules capable of catalyzing steps in their own respective syntheses. Competition would result in selective propagation of the more efficient reflexive catalysts, and the accelerated consumption of starting materials would gradually extinguish inefficient or uncatalyzed competing reactions. This can be demonstrated in a mathematical model and appears to be subject to test in relatively simple, though long term, laboratory experiments.

The concept of reflexive catalysis applies not only to reactions in solution. It can, for instance, be extended to include such physical effects as the stabilization of a colloid droplet by a molecule being synthesized in that droplet. Thus provided with natural selection on a molecular level, Oparin's description of the origin and early evolution of protoplasm becomes reasonable.

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PHENOCOPIES AND GENOTYPE, WITH SPECIAL REFERENCE TO SPORADICALLY-OCCURRING DEVELOPMENTAL VARIANTS[1]

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In extensive and beautiful experiments Goldschmidt demonstrated in 1935 that the exposure of Drosophila pupae to heat shocks led to the appearance, among the imagoes, of a wide array of morphological variations and defects. The great majority of these non-hereditary phenodeviants were close mimics of the phenotypes of known mutants. For these experimentally-produced abnormalities Goldschmidt introduced the term 'phenocopies'. He found that the extent and manner of response to heat shocks varied with the developmental stage at the time of treatment, the duration of exposure, the intensity of heat, and the hereditary constitution. Various stocks differed consistently in the nature of their response, but no relation was observed between the phenotypic traits or other genetic peculiarities of a particular stock and the kinds of phenocopies that stock was likely to yield.

Hereditary traits are frequently also mimicked by spontaneously-occurring variations of an apparently non-genetic nature. A wide spectrum of such sporadic variants is commonly found in most types of organisms. Our present discussion will be concerned with the relations between sporadic phenodeviants and experimentally-produced phenocopies on the one hand, and, on the other, with the meaning of these phenomena in terms of their bearers' genotype.

RUMPLESSNESS IN FOWLS

Let us start with a brief discussion of rumplessness of fowl. This is a well-known genetic trait, represented by two independent mutations, but it is also found relatively often as a sporadic phenodeviant and, in addition, occurs in response to many experimental treatments of early developmental stages. Hereditary rumplessness exists as a dominant and as a recessive mutation; multiple modifying genes may in similar ways alter the morphological expression of both forms of hereditary rumplessness, producing conditions intermediate between complete absence of the tail and its normal conformation (Dunn, 1925; Dunn and Landauer, 1934, 1936; Landauer,

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1945). Instances of sporadic rumplessness have been detected in every breed and stock that has been examined for it. When adequate samples were studied, it was found that the incidence of sporadic rumplessness remained constant within a particular stock, but that it differed significantly between stocks and breeds. To give some specific examples, the mean incidence of sporadic rumplessness among survivors of the 13th day of incubation has, in our experience, been 0.5 percent for Jungle fowl, 1.6 percent for White Leghorns and 5.7 percent for Silver Gray Dorking fowl. It is of interest to note that in the stocks with relatively low incidence of sporadic rumplessness (Jungle, Leghorn) the defect is invariably complete, i.e. all tail vertebrae are lacking, whereas in the stock with highest incidence (Dorking) intermediate conditions, with the presence of rudimentary and fused tail vertebrae, occur. A statistical analysis of data for White Leghorn and Silver Gray Dorking fowl has demonstrated that in regard to the frequency of rumplessness in their progenies significant heterogeneity between mothers obtained in both populations. Yet, we failed in attempts to raise the incidence of sporadic rumplessness by selection, and it is likely that, as in the case of taillessness of rats (Dunn, Gluecksohn-Schoenheimer, Curtis and Dunning, 1942), the incidence among progenies remains the same irrespective of the parental phenotypes.

Next, we were interested to determine if relations exist between the incidence with which sporadic rumplessness occurs in a particular stock and the response of that stock to rumplessness-inducing teratogenic compounds. Our experiments with insulin, boric acid, pilocarpine and yet other substances have shown conclusively that the mother's genotype influences the results. Within a particular stock we found significant heterogeneity between mothers in the likelihood of obtaining rumplessness by treatment of their developing embryos; also, selection within a breed for low or high response to rumplessness-inducing compounds was quite effective. Great response dissimilarities obtained between breeds, and these breed differences paralleled those in incidence of sporadic rumplessness, that is, the higher the frequency of sporadic rumplessness in a particular stock the greater was its response to rumplessness-inducing compounds (Landauer 1948, 1954, 1956; Landauer and Bliss, 1946). The same relation held between intrabreed differences in the occurrence of sporadic rumplessness and the response to phenocopy-producing agencies; and this was true not only for treatment with various chemicals, but also for mechanical shaking (Landauer and Baumann, 1943).

The second question that interested us was whether, in addition to the relationship between incidence of sporadic rumplessness and response to rumplessness-inducing teratogens, there was also common ground between the tendency of a particular stock to produce sporadic rumplessness at a relatively low or high rate and the hereditary transmission of rumplessness. A good opportunity for putting this question to a test was offered by the fact that penetrance and expressivity of the recessive rumpless mutation (rp-2) are relatively low, at least in combination with the residual heredity

of our strain. Our stocks with highest and lowest incidence, respectively, of sporadic rumplessness were the Silver Gray Dorking and Jungle fowl families previously mentioned. The recessive rumpless mutation was introduced into these two breeds and the resulting F_2 progenies yielded the information shown in table 1 (Landauer, 1955).

TABLE 1

PENETRANCE AND EXPRESSIVITY OF RECESSIVE RUMPLESSNESS (rp-2) IN THE ORIGINAL STOCK AND IN F_2 PROGENIES OF CROSSES TO SILVER GRAY DORKING AND JUNGLE FOWL

	Penetrance %	Expressivity %
rp-2 inter se	32.9	20.8
F_2 (Dorking x rp-2)	40.7	53.4
F_2 (Jungle x rp-2)	7.8	18.8

Penetrance and expressivity of recessive rumplessness showed highly significant differences between the F_2 progenies; both crossbred groups also deviated significantly from the original rp-2 stock in penetrance of the mutant gene; expressivity was greatly raised in the F_2 from crosses to Dorking fowl, but was not significantly altered in the (Jungle x rp-2) F_2 group. The direction of all these differences was such as would be expected if the stock-typical incidence of sporadic rumplessness among Dorking and Jungle fowl had influenced the penetrance and expressivity of recessive rumplessness in a corresponding fashion. We suggested, therefore, that the occurrence of sporadic rumplessness is brought about by genetic factors, of an undetermined nature, which find expression only in relatively rare developmental situations (or, conversely, that, except for relatively rare events, the existing genetic equilibrating mechanisms suffice to guard against abnormality of tail development), but that these factors may become incorporated into the multifactorial genetic system which regulates penetrance and expressivity of recessive rumplessness. From the evidence discussed previously, it appears, furthermore, that these same factors enhance or reduce, as the case may be, the effectiveness of rumplessness-inducing teratogenic compounds. It will be the principal purpose of our further discussion to establish if we are dealing with a situation of more general validity.

OTHER CONGENITAL DEFECTS

Cross-beak is another developmental defect that occurs in many stocks. In our White Leghorn fowl 0.20 percent of all survivors of the 13th day of incubation have shown a cross-beak, whereas among our Black Minorca fowl the corresponding incidence has been 1.63 percent. The difference between the two stocks amounts to 1.43 ± 0.43 percent and is highly significant. The majority of cross-beaked individuals die in late stages of

development, but a few hatch and some of these may reach maturity. The cross-beak condition differs from sporadic rumplessness by clearly having a multifactorial recessive background, with low penetrance and variable expressivity. Affected birds mated to each other produced up to 50 percent cross-beak progeny (Landauer, 1938–39 and unpublished data). A cross-beak condition can be produced experimentally by injecting ethyl carbamate into the yolk sac of developing eggs at any time during the first six days of incubation, and this seems to be the only effect which ethyl carbamate has on chicken embryos. When we compared the Leghorn and Minorca stocks by injecting 40 mg ethyl carbamate into eggs during the first 120 hours of incubation we obtained the results shown in table 2 (Landauer, 1956 a).

TABLE 2

THE INCIDENCE OF CROSS-BEAK IN WHITE LEGHORN AND BLACK MINORCA EMBRYOS AND CHICKS AFTER TREATMENT WITH 40 MG/EGG ETHYL CARBAMATE. SPONTANEOUS INCIDENCE: WHITE LEGHORN 0.20%, BLACK MINORCA 1.63%

Time of treatment	White Leghorn %	Black Minorca %
0–48 hours	6.8 ±1.2	25.4 ±2.1
72–120 hours	7.4 ±1.2	13.5 ±1.8

The data demonstrate clearly that in the stock with the higher incidence of sporadic cross-beak ethyl carbamate was much more effective in producing the same condition experimentally. It is of some interest to note that the two stocks differ in the opposite direction in regard to the incidence of sporadic rumplessness, that is, the latter abnormality is found more commonly in Leghorns than in Minorcas, but since ethyl carbamate has no apparent affinity to the tail structures, this dissimilarity had no influence on the results of treatment. Examples similar to those related for rumplessness and cross-beak could be multiplied from our experience.

An interesting situation of a related nature has been revealed by studies of Warren (1937) and Hyre (1955) on a condition of growing chickens known as "crooked keel". The abnormality derives its name from a deformation of the sternal carina. The facts that interest us here, as related by Warren, are these:

1. Crooked keels occur very rarely if chicks are raised without roosting facilities.

2. Perches with a sharp surface promote the appearance of crooked keel bones; the incidence tends to be the higher the earlier, between 4 and 10 weeks of age, chicks are allowed access to such perches.

3. The incidence of the abnormality is higher in males than in females and also tends to be more extreme in males.

4. It could be shown that hereditary constitutional factors exist which promote the occurrence of the crooked-keel condition. In identical envi-

TABLE 3

THE OCCURRENCE OF CONGENITAL DIAPHRAGMATIC HERNIA IN NEWBORN RATS AS A SPORADIC, STOCK-CONSTANT TRAIT AND AS DETERMINED BY VITAMIN-A DEFICIENCY OF THEIR MOTHERS

Stock	Sporadic incidence %	Incidence after vitamin-A deficiency %
Albino	0	0.9
D.H.	2.7	19.0

ronments stocks differ markedly in incidence. Within stocks selection for or against occurrence of the abnormality is effective.

5. In the presence of dietary deficiency in vitamin D the chances for the occurrence of crooked keels are greatly enhanced.

It follows from these observations that under natural conditions crooked keels develop only as a rare sporadic defect, that the incidence varies between different stocks and breeds, that it has a complex genetic basis, and that its appearance may be greatly promoted by certain conditions of the environment.

Andersen (1949) reported that in pregnant rats nutritional vitamin-A deficiency is responsible for the appearance of congenital diaphragmatic hernia among their young. This defect also occurs as a sporadic phenodeviant, and we find again that the stock-typical sporadic incidence influences the response to experimental conditions (table 3).

Congenital harelip and cleft palate are found in certain stocks of mice as a sporadic syndrome. Experiments with two such stocks have yielded results of particular interest (Fraser, Fainstat and Kalter, 1953; Fraser, Kalter, Walker and Fainstat, 1954; Kalter, 1954; Walker and Fraser, 1954, 1956). The relevant data from Fraser's laboratory are reproduced in table 4. The sporadic incidence of harelip and cleft palate was maintained at a constant rate during prolonged close inbreeding. When pregnant mothers from these two stocks were treated with cortisone, many of the newborn young

TABLE 4

THE SPORADIC INCIDENCE OF A SYNDROME OF HARELIP AND CLEFT PALATE IN TWO STOCKS OF MICE MAINTAINED BY BROTHER-SISTER MATINGS AND THE FREQUENCY OF CLEFT PALATE PRODUCED IN THESE STOCKS BY TREATMENT OF THE PREGNANT MOTHERS WITH CORTISONE ACETATE (2.5 MG/DAY FOR 4 DAYS, BEGINNING WITH THE 11TH DAY). CASES OF COMBINED HARELIP AND CLEFT PALATE, PRESUMED TO BE OF SPORADIC ORIGIN, WERE OMITTED FROM THE EXPERIMENTAL GROUPS

Stock	Generations of inbreeding	Sporadic incidence (harelip & cleft palate)	Incidence after cortisone treatment (cleft palate)
A/Jax	88	5.0	100
C57Bl/6Jax	45	0.2	18.7

showed a cleft palate, the frequency of this defect being much higher in the stock (A/Jax) with the more common sporadic occurrence of the harelip and cleft palate syndrome. Embryological investigations have shed some light on the specific response of palate development to cortisone treatment. It was found that in A/Jax embryos the secondary palate normally closes at a later developmental stage than in C57Bl embryos. Starting cortisone treatment of C57Bl females earlier in pregnancy did not raise the incidence of cleft palate among their young above that obtained in somewhat later stages. Cortisone treatment produced a retardation in the movements of the palatine shelves, and this delay was much more marked in A/Jax than in C57Bl embryos. It is clear, therefore, that cortisone intervenes in a specific manner in the processes of palate development, and the incidence of the resulting disturbances varies with the stock-typical tendency for the production of sporadic harelip and cleft palate. As in chickens, relations between the occurrence of morphological variants as relatively rare sporadic events and the ease of producing the same variants as phenocopies appear to be a common phenomenon in inbred stocks of mice (Runner, 1953).

PARTHENOGENESIS IN THE SILKWORM

For our last example we turn to insects, specifically to the problem of natural and artificial parthenogenesis in the silkworm, *Bombyx mori*. There is an extensive literature on the subject, but our discussion is chiefly based on publications by Jucci, Niceta, Wen, Astaurov, Umeya and Horada, and Fukuda. The facts that concern us here may be briefly stated as follows:

1. Spontaneous parthenogenesis occurs, with varying frequency, in all races of *Bombyx mori*. It is relatively rare in univoltine races, i.e. those with only one generation annually, more common in bi- and polyvoltine races.

2. In univoltine races spontaneous parthenogenesis rarely proceeds beyond the earliest stages of segmentation; in bi- and polyvoltine races natural parthenogenesis frequently continues to the end of embryonic development, the embryos generally dying at that time, but viable larvae on occasion emerging from the eggs.

3. The eggs of different mothers, belonging to one and the same race, show significant heterogeneity in the incidence of spontaneous parthenogenesis. The same holds true for the extent to which such development proceeds. The incidence of spontaneous parthenogenesis can be raised by selection.

4. Artificial parthenogenesis (induced by heat activation, treatment with hydrochloric or sulfuric acid, etc.) succeeds the better the higher in a race is the incidence of spontaneous parthenogenesis. Bi- and polyvoltine races respond much more readily to such treatment than do univoltine races. F_1 eggs from crosses between bivoltine races or between bivoltine and polyvoltine stocks are particularly favorable material for the induction of parthenogenesis, presumably on account of hybrid vigor.

5. Within races, significant heterogeneity was found in the ease with which eggs of different mothers could be stimulated to develop parthenogenetically. Eggs that had been laid by mothers of induced parthenogenetic origin showed an increased tendency toward spontaneous parthenogenesis, a result allied to the "genetic assimilation" of Waddington (1953, 1956).

Since the occurrence of spontaneous parthenogenesis or the degree of response to parthenogenesis-inducing treatment appears to be closely related to voltinism, the following additional facts are of interest[2]. They will at the same time serve as a useful warning against the assumption that we are dealing with a simple situation, genetically or otherwise.

6. It was demonstrated by transplantation of ovaries that a diffusible substance is involved in the determination of voltinism. Subsequently, it was found that secretions of the subesophageal ganglion, which is in turn controlled by the brain, decide in fourth instar caterpillars of the silkworm whether the emerging mothers will produce univoltine or bivoltine offspring.

7. Photoperiodism, i.e. length of day during the incubation period of the eggs, and temperature are important external agencies in the decision whether the resulting moths will lay eggs that give rise to univoltine or bivoltine individuals.

DISCUSSION AND CONCLUSIONS

The evidence which we have discussed is representative of a much larger body of information. It will suffice, I believe, to support the following conclusions:

1. Many sporadically-occurring variants of form or function arise on the basis of a specific hereditary constitution; differences of genotype presumably account within a species for variations in incidence of these phenodeviants or for their non-occurrence.

2. Definite relations exist between the frequency with which particular sporadic variants are found in a stock and the ease of producing the same variants by experimental means, the incidence of induced variants rising -often disproportionately- with increasing incidence of the sporadic type.

3. In multifactorially-determined traits with irregular expression, penetrance and expressivity may be improved by a residual genotype that favors a relatively high incidence of the homologous sporadic variants, whereas residual genotypes of the opposite kind may act in a contrary fashion.

4. The response to agencies inducing a specific phenocopy often varies between stocks of a species. Within a stock selection for greater or lesser response is effective. Factors of maternal physiology often play an important role.

It is well known that within multiple allelic series the phenotype of a particular allele may be shifted, as a consequence of changes in environ-

[2]A recent monograph by Lees (1955) contains a stimulating summary and discussion of the physiological aspects of diapause in silkworms.

mental temperature, so as to resemble more normal or more abnormal members of the series (cf. Goldschmidt, 1938). The early studies of Goldschmidt with heat shocks had shown that, in comparison with the wild-type, the production of phenocopies succeeded more readily in organisms which were heterozygous for a recessive mutant gene with homologous morphological effects, and this has been abundantly confirmed, most recently by Sang and McDonald (1954) in their experiments with sodium metaborate. These and similar examples are instances in which single-gene substitutions influence homologously the outcome of phenocopy experiments. It seems likely that gradual transitions lead from these situations to the genetically-unanalyzed conditions which are responsible for the occasional appearance in particular stocks of sporadic variants, conditions which also promote the homologous response to phenocopy-inducing agencies.

It is clear that the various sporadically-occurring phenodeviants are a heterogeneous group as far as their hereditary background is concerned. Some, such as the crossbeak condition of fowl, are polyfactorial recessives with low penetrance and expressivity. In others, an original single mutation may have become inoperative as a consequence of accumulated plus modifiers; in cases of this kind the mutant phenotype may in rare instances make an appearance as a sporadic variant, while a more or less regular transmission of the trait becomes manifest only after some of the modifiers have been removed or made heterozygous. Such a situation seems to have existed in the cyclopia lethal of fowl (Landauer, 1956 b). Many of these conditions are probably quasi-continuous variations in the meaning Grüneberg (1952) has given to this term. Forces of the environment may during development inhibit the activity of specific suppressor genes, as has been shown by Glass and Plaine (1952) for Drosophila mutants, and this may be one of the important mechanisms in the origin of phenocopies.

Disturbances of the proper harmony of developmental rates or the intervention of exceptional environmental conditions in the normal functioning of physiological thresholds (or a combination of such events) are unquestionably important factors in the origin of sporadic phenodeviants and of phenocopies. In a recent discussion of preaxial polydactylism of the hind limbs in the mouse Truslove (1956) said: "One gets the impression that there exists in the mouse an embryological situation which is liable to give rise to this anomaly. Some strains are deeply below the threshold and rarely or never produce polydactyls; other strains are nearer the threshold and show sporadic cases, and this applies, for instance, to the CBA inbred strain (Grüneberg, unpublished). Minor genes in unison, and major genes unaided, can lift a mouse embryo over that threshold and thus produce polydactylism." It follows from our discussion that this conclusion applies as well to phenocopy-inducing agencies, and that, in fact, the effectiveness of these external factors is determined by the genetic setting of the threshold mechanism. Truslove concluded her statement by saying: "The mere fact that so many different genes can evoke so similar an effect seems to indicate that they all trigger off the same mechanism; in other

words, that their effect on polydactylism is completely unspecific". As so often in biological discussions, it is difficult to know just what is meant by "unspecific". The effect is obviously quite restricted topographically; hence, one may conclude that, in terms of developmental physiology, differentiating mechanisms are at work which, for example in contrast to the forefeet, predispose the hindfeet to respond; but it may well be, of course, that regional processes of a general nature, such as growth competitions, are at work.

Goldschmidt (1955) believes, in explanation of the origin of phenocopies and with reference to gene-determined substances produced by homologous mutants, that it is "probable that the kinetics of a chain of reaction, elaborating such active substances, is influenced in such a way that velocities of processes, quantities of products, relative timing of collaborating reactions, and concentrations amounting to threshold settings will be the main factors that are being shifted in plus or minus directions." He concedes that "it is quite conceivable that in the phenocopic experiment the shock interferes with the velocity of some reaction by affecting its kinetics directly—, while in the mutation the same effect upon the velocities is produced by a more remote change—." There is no reason to doubt the justice of this interpretation. If, however, we resist the temptation of interpreting phenocopies in terms of their similarity to single-gene events, even if mutants inviting such comparisons (as for example dominant rumplessness) are available, it may turn out that the distance between the phenocopy-inducing factors and the conditions precipitating the spontaneous origin of identical variants is less great than one would otherwise need to assume. For it is clear from the examples which have been discussed that the residual genetic factors which set the stage for sporadic developmental "accidents" also predispose, and in a correlated manner, toward the phenocopy response. The fact that both situations are similarly affected by plus or minus modifiers, strengthens the conviction of their being closely related.

Paul Ehrlich's dictum that *corpora non agunt nisi fixata* may well be applied to the relations between a developing organism and its environment. The environment becomes in some fashion incorporated into the living system before that system reacts. But, barring sledge hammer procedures such as exposure to heavy doses of X-rays, it is the nature of the reacting system that decides what response will follow a given insult. In the case of sporadic variations and induced phenocopies these reactions, as far as they have been observed, do not differ in nature from those encountered in the presence of ordinary mutant genes.

It is presumably no coincidence that the kinds of phenocopy effect which can be most readily obtained, and often by a multiplicity of means, are variants which also occur as a consequence of more than one gene substitution. It is well recognized that the development of every part and organ, the maintenance of all vital functions is controlled, if often in a round-

about fashion, by multiple genes, thereby providing dynamically equilibrated safeguards. The occurrence of several independent mutations with similar phenotypic effects (such as rumplessness and micromelia in fowl) is presumably evidence for the conclusion that the corresponding normal sequence of developmental events is in precarious equilibrium, and this may well be true for more than one link in the chain of the particular developmental events. In the same sense our evidence leads us to conclude that sporadic defects as well as experimental phenocopies are the results of events through which ordinarily hidden weaknesses of developmental equilibria become manifest and that these weaknesses have a definite, if complex, genetic basis. If the phenocopy concept in its narrow meaning of a purely environmental interference with developmental processes must be abandoned, it is clear that the existence of crypto-genes and their spontaneous or experimental liberation confront us with many new problems.[3]

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[3]After the present paper was written there appeared an important publication by R. B. Goldschmidt and L. K. Piternick, New experiments on chemical phenocopies. Proc. Nat. Acad. Sci. 42, 299–304, 1956. Supporting evidence can also be found in an interesting recent publication by M. Bertschmann: Versuche zur phänokoperienden Wirkung von Chemikalien (Nitrogen-Mustard U.A.) bei *Drosophila melanogaster*. Zeitschr. ind. Abst. Vererb. 87, 229–266, 1955.

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DISCUSSION OF PROFESSOR LANDAUER'S PAPER

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Dr. Landauer has presented a very interesting group of facts and conclusions, which belongs to a rather neglected field of genetics. In classic genetics 100 percent penetrant and expressive mutants were marked as "most useful" presupposing that genetics is identical with the study of crossing over. Simultaneously rare abnormalities, which could not be extracted as simple mutants or, at least, selected for successfully, were only noted as freaks, of no interest to genetics. Only a few geneticists realized that the low penetrant mutants and the apparently non-hereditary freaks may be excellent material for the study of physiological genetics. Therefore I am glad that Dr. Landauer has drawn attention to such facts as promise an efficient approach to the study of genic action via a comparison of phenocopic and mutant action. It happens that we have been studying the same problems in Drosophila with a very similar approach. Our results and conclusions largely agree with those presented today and, perhaps, lead a little farther.

Let us begin the discussion with what the speaker calls sporadic defects ressembling known mutant conditions. He finds that their percentage incidence is typical for specific genetic lines and concludes that they are based upon a probably complex genetic situation which pushes a precariously balanced and therefore sensitive developmental situation beyond a threshold for normal development. In Drosophila we have found many facts which, while agreeing with this statement, permit us to make it more positive in genetic terms. We. studied mutant conditions based upon a major locus and a number of smaller ones which in the least penetrant members of a series appear like nonhereditary sporadic variations with an incidence of less than one percent. The hereditary nature is proven by selection of a homozygous line breeding true to such a low incidence or a similar one, which resists further selection. Thus we are dealing really with a mutant—or, more correctly, a polygenic mutant effect—of lowest penetrance. The podoptera effect (a leg like structure instead of a wing) is an example. But from different genetic strains lines can be isolated with higher penetrance, and finally lines in which by selection a 100 percent penetrance can be isolated. Thus we have all quantitative gradations between an apparent chance effect, apparent if no special analysis is made; a typical low penetrant mutant and a full penetrant mutant, or polygenic mutant conditions. Shall we, then, speak of sporadic developmental variants? I do not think

so, as we can prove that these variants are based upon a group of mutants, both major and minor ones, some of which we could isolate as located in individual chromosomes. The sporadic variant is thus genetically what I called a mutant condition, obtainable in homozygous form, based usually upon multiple loci, collaborating to a plus-minus low penetrant phenotype. Thus from the point of view of physiological genetics we have a strict parallel to a series of multiple allelic conditions as found for example in the well known vestigial series, with the only difference being the genetic causation by a multifactorial instead of a multiple allelic series. Thus we see that the phenomenon may be described, as the speaker did, as a developmental effect which takes place if a definite setup of modifiers is available. But I think it more correct to describe the genetic facts directly as a polygenic mutant condition of specific but low penetrance; thus permitting to us fit them into a whole series from almost not recognizable hereditary status up to complete penetrance. This description allows also for the possibility that a single preponderant locus in the polygenic setup mutates to such a high potency that its action outdistances that of the weaker modifiers and controls alone a totally penetrant effect, thus appearing as a single mutant.

The second group of facts emphasized by the speaker is the enhancement of penetrance in a mutant stock of incomplete penetrance if simultaneously the genetic background for a high incidence of sporadic variants is present. There is a parallel to this in the podoptera work. When two genetically different podoptera types are crossed the heterozygotes show a podoptera effect just as if the podoptera factors were allelic. We spoke of spurious allelism; one could also speak of mutual epistasis. The parallel to the chicken experiments is the collaboration of two different genetic systems affecting the same developmental process. In a more simple form the collaboration of different mutants toward enhancing the same phenotype illustrates the same principle.

The third important feature emphasized by the speaker is the different sensitivity of different genetic lines to the production of phenocopies by chemical treatment and the parallelism of the grades of sensitivity with the amount of presence of genetically controlled sporadic variants in the line. This theoretically important fact seems rather expected when, according to the foregoing discussion, the genetic base for sporadic variation is actually a mutant condition for simple or polygenic control of the specific phenotype, acting with low penetrance. It is then the action of the phenocopic agent to enhance the penetrance, with the, to me, amazing consequence that the phenocopy turns out to be in such cases nothing but the enhancement of an already present genetic condition. This might be a polygenic setup. But in another case it could be a low penetrant multiple allele. In our new work with sodium borate induced phenocopies in Drosophila a number of facts were found which point to the possibility that all phenocopic effects are actually based upon the presence of a genetic condition which is not necessarily a special, favorable set of multiple modifiers affecting the opening

or closing of pathways of development but rather the presence of specific mutants with subthreshold action, sometimes called isoalleles. We find, like Landauer, genetic conditions for the quantitative response to phenocopic action, which can be selected for. We find further a surprisingly large genetic control of the qualitative response. About 20 phenocopies were typically produced, by the same chemical treatment, some of them, or sometimes a single one, characterizing a definite wild stock (always after the same treatment). Thus each stock produced one or more phenocopies as its specific response; specific also was the preponderance of one or the other effect and, in some cases, a single rare phenocopy was specific for only one special line. All this may be understood under a genetic and developmental scheme as developed by Landauer at the end of his paper. But a group of further facts point strongly to the idea that quantitative and qualitative specificities of phenocopic action in different stocks, the specific effects upon penetrance and the relations to so called spontaneous variants, are all based upon the presence everywhere of an array of subthreshold mutants, sometimes varying beyond the threshold of visible action and capable of being shifted above it by phenocopic action when the proper background of genetic modifiers is present. Some of these facts are: A heterozygote, (if the explanation is correct, actually a compound) between a mutant and a wild line with specific tendency for the phenocopy of the same mutant, may exhibit a small penetrance of the character (a little dominance) and this in proportion to the sensitivity for phenocopic response of the line used. If the "compound" is treated with the phenocopic agent a high reaction occurs (which could be described as a change of dominance), which again is proportional to the degree of sensitivity of the wild type line involved. A second group of facts is the enhancement of the expressivity of mutant effects by phenocopic treatment, though in other cases the opposite happens. Very important is the fact that the low penetrance of a mutant may be considerably increased by the same treatment. Still more surprising are the experiments with mutants which have lost all expression, probably by accumulation of plus modifiers. The phenocopic treatment brings out the expression.

Nearly related and still more impressive is the following group of facts: Spineless is an allele of aristopedia but without any effect upon the arista. When spineless is treated with borate a high percentage of rather strong aristopedia appears, much higher than the phenocopic production of aristopedia in wild stocks. Amherst is one wild stock for which an unusually high incidence and also expressivity of aristopedia phenocopies is specific. A "compound" of spineless and Amherst treated with borate gives a still higher aristopedia effect than that of either parent line!

But in spite of all these highly suggestive results we are not ready yet to consider the presence of subthreshold mutants as the final explanation of all the facts under discussion. The reason is that we (Goldschmidt and Piternick) have a few results, not to be detailed here, which do not fit the

expectations; and further that some crucial experiments have not yet been finished. Therefore the facts and tentative conclusions are presented with due caution. One thing however seems certain: that the subject of Landauer's work and report turns out to be a most important tool for the analysis of gene action and the process of mutation and that the final outcome of the analysis is bound to influence strongly both physiological and population genetics.

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THE DEVELOPMENTAL MECHANISMS OF GENES AFFECTING THE AXIAL SKELETON OF THE MOUSE*

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INTRODUCTION

There is a general tendency in present-day biology to regard morphology with disdain and to expect salvation from biochemistry. This is to some extent understandable in those fields of biology where a solid body of knowledge about structure has been accumulated and where one wants to advance from that safe basis to the dynamics of the molecular level. It is a very curious fact that this attitude should also prevail in the field of genetics. For, although the morphological basis of gene effects is still almost unknown, many people seem to think that genetics is a privileged domain in which, by some dispensation of providence, the morphological level can be skipped and a direct step be done right into the realm of biochemistry. This may be possible in the case of microorganisms which cannot boast of much structural diversity. But there is no short cut, no *Via regia*, where we are dealing with structure. Therefore, unless we are prepared to ignore what constitutes 90 per cent of organic diversity and, to my mind, 90 per cent of its fascination, we shall have to lay the morphological foundations, painful though that may be, and to discover first what exactly needs an explanation before we start with the business of explaining.

SOME FEATURES IN THE NORMAL DEVELOPMENT OF THE AXIAL SKELETON

This paper deals with a number of inherited abnormalities of the axial skeleton of the mouse. All of them affect the tail to a greater or lesser extent, and in the living animal some of them show a superficial resemblance to each other. For reasons of time, it will not be possible to discuss all the conditions of this group which have been described; in some cases, little or nothing is known about their structure and development. I shall mainly talk about a small group of genes with which I am familiar from my own work, but I shall also mention briefly some of the important contributions in this field which have been made by Dunn and his associates.

Before describing the various mutant genes and their effects, it will be convenient to recall a few major events which happen during the normal development of the vertebral column in mammals. In nine-day old mouse em-

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bryos, the anterior limb buds are represented by long-drawn-out swellings, but there is no sign yet of posterior limb buds or of a tail. During the next 24 hours, a considerable elongation of the axial structures takes place; a tail bud makes its appearance in which one can distinguish the neural tube, the notochord, the tail gut and the paraxial mesoderm. It is commonly believed that in the elongation which takes place, the notochord plays a leading role. The paraxial mesoderm is not yet segmented in the tail. Segmentation or somite formation spreads into the tail from the trunk region, and as as the tail continues to grow near the tip, so somite formation follows it at a distance, and in the end, all but a small remnant of the paraxial mesoderm has been transformed into about 30 caudal segments. The somites which are separated from each other by intersegmental vessels are soon further transformed by processes which start anteriorly and thence spread in a caudal direction. The ventro-medial regions of the somites disintegrate, and the cells so liberated migrate towards the midline and join with those of the opposite side round the notochord and underneath the neural tube. The material between successive intersegmental vessels thus formed by fellow somites constitutes a sclerotome which at first has a uniform tissue density and no other visible differentiations. Very soon, and again spreading in an antero-posterior direction, the successive sclerotomes differentiate into anterior and posterior sclerotome halves; these differ by their cell density; the anterior sclerotome halves are of a comparatively low tissue density while in the posterior sclerotome halves the cells are much more densely packed. At this stage, anterior and posterior sclerotome halves are separated from each other by a transitory fissure, the fissure of von Ebner or the sclerotomic fissure. The definitive segments are bordered by two successive sclerotomic fissures and thus include material from two successive primitive segments. The sclerotomic fissure at first sharply separates low tissue density in front from high tissue density behind. Soon, however, this arrangement is modified by the appearance of a region of higher density in front of the sclerotomic fissures while a region of lower cell density invades the posterior sclerotome halves from behind. The whole somewhat complicated process leads to the separation of materials which will form the vertebrae themselves from materials which will contribute to the formation of intervertebral discs. Once this process has been accomplished, histogenesis supervenes, and the materials set aside for the vertebrae chondrify while the material which will become incorporated into the intervertebral discs becomes largely (though by no means entirely) fibrous. The later stages of histogenesis leading to ossification need not be described here as the genes in question do not affect these late stages of development.

UNDULATED

The recessive gene for undulated (*un/un;* Grüneberg, 1950, 1954) produces characteristic effects from one end of the vertebral column to the other. The number of vertebrae is normal. The tail is shortened to about

80 percent of its normal length, and the individual tail vertebrae are very characteristic in that their anterior ends are normal while their posterior ends are defective; as a result, tail kinks are generally present in addition to the shortening of the tail. More anteriorly, in the lumbo-sacral region, the vertebrae are not shortened, but show a reduction in size of the bodies in a dorso-ventral direction which is often accompanied by ossification from bilateral twin centres. Transverse and other processes are either reduced in size or missing. Fusions between adjacent sternebrae which are often present are apparently mediated by a reduction in length of certain ribs. Otherwise, the only anomaly outside the axial skeleton is the fact that the acromion scapulae is not represented by bone, but by a ligament.

The first sign of abnormality which has been detected occurs in 11-days old embryos. At that stage, the condensation of cells cranial to the sclerotomic fissure is not as marked as in normal embryos and it is less distinct from the condensed tissue at its back. According to one embryological view which we strongly support, the condensation cranial to the sclerotomic fissure contributes towards the formation of the centrum of the vertebra, the transverse processes, neural arches and the ribs. This material seems to be reduced in quantity and tends to be retained with the condensed tissue behind it which forms the intervertebral discs (other than the nucleus pulposus which is mainly derived from the notochord). It can in fact be seen in undulated mice that while the vertebrae are reduced in size, the intervertebral discs are thicker than normal. It thus appears that the anomaly of undulated mice is basically a faulty distribution of materials as between vertebrae and intervertebral discs, the latter being increased at the expense of the former. This happens during the later differentiation of the sclerotomes but long before chondrification.

TAIL-KINKS

Tail-kinks (symbol *tk;* Grüneberg, 1955a) is a recessive gene which has striking effects at the two ends of the vertebral column while the intervening region is but slightly affected. The number of vertebrae is normal. The cervical and upper thoracic vertebrae are in a chaotic state; many of them are present in the form of several separate ossicles, and such elements belonging to adjacent vertebrae may be fused with each other. The ribs are often abnormal in various ways, and often there are fusions between sternebrae which are obviously secondary to the rib abnormalities. The lower thoracic region is more nearly normal, and in the lumbo-sacral vertebrae no abnormalities are found except an occasional absence of a transverse process. Abnormalities again occur in the caudal vertebrae where they increase in severity in a distal direction.

Embryologically, the anomalies can be traced back to a stage in the membranous skeleton earlier than that affected by the undulated gene. When in the normal embryo the sclerotomes become differentiated into sclerotome halves of differing tissue density, this differentiation is delayed and re-

mains irregular in *tk/tk* embryos; in the cervical region, this defect is recognizable in 10-day embryos; no abnormalities have been discovered in still earlier stages. It seems that only sclerotomes derived from small somites at the two ends of the vertebral column are involved while the sclerotomes derived from the big somites in the lumbo-sacral region are virtually immune.

BENT-TAIL

In this sex-linked semi-dominant gene (symbol *Bn*; Garber, 1952; Grüneberg, 1955b), the shortening of the tail in hemizygous males is partly due to a reduction in number of the tail vertebrae by about four or five elements. In addition, the tail vertebrae are progressively shorter than normal in a distal direction. The smallest of the caudal vertebrae tend to ossify from bilateral twin centres. The basic anomaly (which has, in this case, been inferred from the adult state only) seems to be a reduction in the growth of the tail bud. As the tail seems to grow less, or more slowly, than in a normal embryo, increasingly smaller segments are formed and in the end the growth potencies of the tail are exhausted prematurely so that the total number of tail segments formed is reduced. There is no evidence in this mutant that parts of the tail have been lost secondarily.

As in Bent-tail the number of tail vertebrae is reduced (at least in the males), it is clear that a developmental process preceding the formation of sclerotomes is involved. It is not clear, however, whether segmentation as such is at fault, or whether segmentation is abnormal as a result of a reduced growth of the tail bud.

BRACHYURY AND TAILLESSNESS

The anatomy and embryology of the vertebral column in Brachyury ($T/+$) and in taillessness (T/t^n) has been worked out in some detail by Dunn and his associates. Confining ourselves to the viable heterozygotes, it seems that in $T/+$ embryos the tail at first shows about normal growth and segmentation, except that the notochord is abnormal in parts of the tail and elsewhere; some anomalies of the neural tube are probably secondary in nature. The distal region of the tail is later separated from the proximal part by a constriction and is ultimately lost (Chesley, 1935). The notochord does not extend into the free tail in tailless (T/t^n) embryos which show the constriction at the root of the tail (Gluecksohn-Schoenheimer, 1938).

It has been generally assumed that in the outgrowth of the tail, the notochord plays a leading role. The case of Brachyury and taillessness shows that the tail of the mouse can grow beyond the point at which the notochord ends. Whether the total number of segments formed by $T/+$ embryos is normal is, however, not known. In any case, the notochord is not the only factor which determines the extent of the outgrowth of the tail. This gives added interest to a situation which has recently been discovered in the mutant gene vestigial.

VESTIGIAL-TAIL

In this recessive condition (Heston, 1951; Grüneberg, 1957) the tail is either completely absent or reduced to a short stump. The tail bud in 10-day embryos is about normal in size. At the age of 10½ days, the tail bud is somewhat smaller than normal, and at 11 days the difference is obvious. At the age of 12 days, constrictions of the tail begin to separate the rudimentary tail bud from the rump, and at 13 days, a condition very much like that at birth is reached, with either no tail at all, or a thin tail filament, or a short tail stump. In sectioned material, the notochord is completely normal; the tail gut is slightly reduced. The neural tube is at first normal, but it fails to undergo the gradual reduction in size which, in normal development, leads to its ultimate disappearance from the tail. In later stages, the neural tube undergoes very striking changes such as a splitting off of parts of its ventral region; later these separated parts usually develop secondary lumina.

In the course of the work on vestigial-tail, a structure was noticed in normal tail development which seems to have escaped the attention of previous workers. In embryos of 10½ days, there exists a thickening of the ventral tail ectoderm under the tail tip which in its structure resembles very much the well known apical ectodermal ridge of the limb buds in mammals and birds. This 'ventral ectodermal ridge of the tail' (Grüneberg, 1956) gradually disappears as the segmentation of the tail is completed, this process being virtually complete in 12-day embryos. The origin of the structure can be traced to the cloacal membrane. Here, where entoderm and ectoderm come into direct contact with no mesoderm in between, the ectoderm assumes a columnar structure. This columnar structure spreads along the ventral surface of the tail until it reaches the tip of the tail bud; as it spreads in a distal direction, it loses contact with the cloacal membrane so that at the height of its development, the ventral ectodermal ridge is separated from the region of the cloacal membrane by ordinary pavement epithelium. Where embryonic ectoderm becomes thickened, it is usually either about to form a structure which will persist in subsequent development, or else it assumes a transitory but specific function, as in the case of the apical ectodermal ridge of the limb buds. That the ventral ectodermal ridge of the tail has a somewhat similar stimulatory function is suggested by its histological similarity to the ridge of the limb buds, by the fact that it is present near the growth zone of the tail, and finally that its existence coincides with the phase of active growth of the tail bud. To this must be added the significant fact that in vestigial-tail embryos, the ventral ectodermal ridge of the tail is reduced to about one quarter of its normal size or less; moreover, it does not occupy the tip of the tail, but is situated some way proximal to it. There is thus strong circumstantial evidence that the ventral ectodermal ridge of the tail is a stimulatory organ necessary for the normal growth and differentiation of the tail, and that the basic anomaly of vestigial mice is the reduction of this organ to a small fraction of its nor-

mal size. On this reading of the facts, the gross deformities of the neural tube in vestigial embryos are to be regarded as secondary. The situation is analogous to that in the wingless gene in the domestic fowl (Zwilling 1949) where absence of the wings is associated with reduction or absence of the apical ectodermal ridge. Unlike the situation in undulated and tail-kinks, where the tail is involved as part of the vertebral column, in vestigial the tail is involved as such by interference with its own stimulatory organ.

THE URO-RECTO-CAUDAL SYNDROME

The discovery of the ventral ectodermal ridge of the tail and its relations to the cloacal membrane is of some interest in connection with another gene in the mouse, Danforth's short-tail (Dunn, Gluecksohn-Schoenheimer & Bryson 1940). In the viable *Sd/+* heterozygote, the tail is reduced to a varying extent. Gluecksohn-Schoenheimer (1945), who investigated the embryology of this genotype discovered that there is a degeneration of the notochord which disappears almost completely throughout its length, and there is a breakdown of greater or lesser parts of the tail. Gluecksohn-Schoenheimer insisted that the breakdown of the notochord had nothing to do with the breakdown of the tail, but failed to discover the reason for the latter. The lethal *Sd/Sd* homozygote is tailless, has an imperforate anus, a persisting cloaca and lacks kidneys. Dunn and Gluecksohn-Schoenheimer (1944, 1947) later discovered that a similar syndrome involving reduction or absence of the tail and abnormalities of the derivatives of the cloaca is produced by a whole series of different genotypes. This syndrome was later named the 'uro-recto-caudal syndrome' by the present author. Dunn and Gluecksohn-Schoenheimer suggested that the 'abnormality arises whenever some process in early development is sufficiently deflected from its normal path and that the different mutations affect this process in different degrees. It can be inferred from the genetical study that the process involves an inductive relationship by which the normal development of the posterior gut and urogenital system is connected with the development of the axial system.' In the light of the facts which I have put before you, it seems a plausible hypothesis that the ventral ectodermal ridge of the tail, with its proven relations to the cloacal membrane, is the connecting link which holds the uro-recto-caudal syndrome together, and whose existence was postulated on purely genetical grounds by Dunn and Gluecksohn-Schoenheimer. Naturally, the embryology of *Sd/+* and *Sd/Sd* will now have to be re-investigated; for it remains to be shown whether the reduction of the tail in *Sd/+* and particularly the absence of the tail in *Sd/Sd* is indeed caused by some abnormality of the ventral ectodermal ridge of the tail as in vestigial. I have started on the collection of the material and I hope to get down to it when I return to England in the autumn. Meanwhile, one is perhaps justified in taking a somewhat more optimistic view about the value of so-called 'purely descriptive methods' in developmental genetics whose potentialities certain authors never seem to tire to belittle.

CONCLUDING REMARKS

The few examples which I have discussed show in a general way how the static 'Mendelian characters' can be replaced by processes which form a definite sequence in development. This, of course, is only a first step towards a dynamic understanding of morphological gene effects. But I venture to suggest that unless this first step is taken, however laborious it may be, we shall never get a proper understanding of the mode of action of those genes which, in the aggregate, have been responsible for evolution. For, without underestimating the importance of evolution on the biochemical level, evolution is essentially a morphological phenomenon.

The sequence in development in which genes act should not be confused with what has often been called the 'time of action' of a gene. In terms of developmental processes, the outgrowth of the tail tip precedes segmentation in that region, which in turn precedes the formation of sclerotomes or their histological differentiation. However, in the embryo, the axial skeleton differentiates in a cranio-caudal sequence. Thus, in an 11-day old mouse embryo, the differentiation of the cervical sclerotomes is sufficiently advanced for the effects of the gene undulated to become detectable; further down the vertebral column, in the lumbo-sacral region, differentiation has gone far enough for the detection of the action of the gene for tail-kinks, and, finally, near the tail tip, the paraxial mesoderm is not yet segmented, and the effects of the vestigial gene may be seen. Although these three genes interfere in processes which follow each other in development, their effects may be simultaneous in different parts of the body because these processes creep along the axial skeleton one after the other, over a considerable period of time.

The whole concept of the 'time of action' of a gene seems to be derived from a totally different field, that of experimental teratology or of phenocopies, as its recent revival has been called. When one subjects an embryo to some treatment, say with X-rays, it is natural to relate the effects produced to the age at which the embryo has been treated. If (and this is a large 'if') the effects happen only during the treatment and not also during an undefined period afterwards, it is legitimate to describe the effects in terms of time of treatment rather than in terms of 'time of onset of treatment'. The facts described in this paper indicate that many genes have no 'time of action' in that sense as the developmental process which the gene affects extends over a long time. Of course it is quite probable that there exist genes which act but once in development and whose localization and timing is more accurately definable.

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DISCUSSION OF PROFESSOR GRÜNEBERG'S PAPER

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I think I can understand Professor Grüneberg's reason for carrying a chip on his shoulder marked "morphological genetics". He is not alone among those interested in morphological problems in regretting that the interest in genes often seems to be exhausted when they have served their purpose as "markers", that is, as indicators of primary elements of structure of the genetic material. Genetic analysis is an essential step in investigating what used to be called the "architecture of the germplasm"; it is the basic method of formal genetics and it has developed into an analytical tool with a resolving power which, as Pontecorvo has recently pointed out, is without equal in biology. It has therefore justified the methods used in developing it, even to using living dynamic structures such as genes or their parts as indicator elements in a spatial system. Both the gene and other elements have survived such indignities before.

But Grüneberg's essential point, I think, is that analysis of *development* can never succeed without a sound basis of morphological description, from which alone the elementary processes and relationships in the developmental matrix can be recognized. With this I think we must agree, particularly when he provides such good examples from his own studies on the development of the mouse of the profit to be derived from descriptive study.

The point of greatest interest to me is Grüneberg's discovery of a structure hitherto unrecognized—the ventral ectodermal ridge of the tail—a deficiency in which, in one mutant (vestigial tail) is accompanied by failure of the tail to complete its development. I agree with Grüneberg that this structure may provide or transmit the inductive stimulus which links into an interdependent developmental system the cloaca and the axial structures. The connection between these has been a long-standing puzzle, for associated abnormalities of the urogenital and gut apertures on the one hand, and the synsacrum and tail on the other hand, have been recognized for a long time in human and mammalian embryology as a unit syndrome. The first step in the analysis of this relationship was the proof that both manifestations were effects of the same genotype, and in one clear case, of a single mutation; the second step was Salome Waelsch's proof of developmental interdependence between axial and urogenital differentiation; now the third step by Grüneberg puts the question in precise form for investigation by morphological methods. One aspect which must certainly be elucidated is the peculiar situation in the most interesting tailless mammal of all, for human embryos undergo first a development and then loss of the tail

which superficially resembles what happens in tailless mutants of the mouse.

The prospects of understanding this set of developmental mechanisms will certainly be improved even though it should turn out, as it so often has in the study of mutants with tail abnormalities, that there is more than one pathway by which the same kind of deviant phenotype is reached. There seem to be many ways of losing a tail, which means I suppose that several processes converge on the important developmental steps by which a tail is acquired.

Since Professor Grüneberg has discovered a structure which had been missed during a century of descriptive study of embryos, it is interesting to reflect on the contrast between such study now and, say, sixty years ago before the influence of ideas from genetics had been felt. One difference is that the perspectives of morphology have been widened and to its basic objectives of understanding development and phylogeny have been added new problems framed as specific causal relations between elements of the hereditary constitution, particular structures, and processes which form the organism into a whole. With the broader purpose comes the better chance that new structures will not only be sought for, but when found will take their place more quickly in a picture already largely outlined by descriptive study.

What we learn in more general terms from this type of analysis is that no single step is a sufficient or terminal one in the study which leads from descriptive morphology to genetics, to morphology again, and now in the immediate future (we hope) to experimental embryology in both its structural and functional forms. The genetical and developmental problems, by such progressions, graduate to new levels which is the best sign of progress in such work.

We must however recognize the long gap between the first appearance of a structure in development and the time when the causal sequences begin. Investigation of this period will certainly require methods additional to morphological ones.

It remains to congratulate Professor Grüneberg for having carried the analysis a good step further, thereby hoisting higher the flag of morphological genetics.

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MEIOTIC DRIVE AS AN EVOLUTIONARY FORCE*

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A heterozygote for alleles *A* and *A'* ordinarily produces gametes carrying each of the alleles with a frequency of 50 per cent. The constancy of allele frequencies from one generation to the next in natural populations of diploid species depends on this equality, which itself depends on the nature of the meiotic divisions. As the study of the genetics of higher organisms becomes more precise and extensive, an increasing number of cases is found in which heterozygotes of certain constitutions fail to produce the two kinds of gametes with equal frequency. Such a pattern of behavior will drastically alter frequencies of alleles in a population; where such a force, potentially capable of altering gene frequencies, is a consequence of the mechanics of the meiotic divisions, we suggest that the name *meiotic drive* be applied. The distinction between meiotic drive and the superficially similar phenomena of gametic selection and gametic competition may be operationally difficult in those instances in which a detailed cytogenetic study cannot be made. The latter, however, represent selection in the ordinary sense, operating in a haploid phase, and as such their effectiveness is directly dependent on the gene content (fitness) of the gametes, whereas the effectiveness of the former is independent of the gene content in the ordinary sense.

HISTORICAL

The first case of meiotic drive was recorded by Gershenson (1928) who noticed that, in certain crosses of *Drosophila obscura* males carrying a special X chromosome found in wild populations, many more females were produced than males, and furthermore, this deviation in sex ratio could not be accounted for by zygote mortality. He argued that, since more X-bearing sperm than Y-bearing sperm were functioning in the fertilization of eggs, this particular X chromosome should rapidly increase in frequency in a population supplanting all other X chromosomes and that the species would then be in danger of extinction because of the scarcity of males. A more detailed cytogenetic study was carried out by Sturtevant and Dobzhansky (1936) with *Drosophila pseudoobscura.* They reported a wide natural distribution of this "sex ratio" X chromosome, and showed that, as a result of abnormal spermatogenesis in males carrying this chromosome, all sperm usually carry an X chromosome (the Y chromosome ordinarily degenerating). They found the phenomenon of "sex ratio" in other members of the *D. obscura* group, including *D. affinis, D. athabasca,* and *D. azteca.*

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The curious meiotic behavior of the "sex ratio" X chromosome seems to depend on a factor (or factors) on that chromosome, and, for this reason, will be referred to as *genic meiotic drive.*

It is found in corn that from plants heterozygous for the abnormal 10 chromosome, which is characterized by a knob on the end of the long arm, approximately 70 per cent of the female gametes carry that knob, whereas the remaining 30 per cent do not (Rhoades, 1942, 1952; Longley, 1945). This "preferential segregation" of chromosomes with knobs is brought about in the presence of the abnormal 10 chromosome by the formation of "neocentromeres" on the knobbed chromosome arms during the meiotic divisions (Rhoades and Vilkomerson, 1942; Rhoades, 1952). The abnormal 10 chromosome responsible for this type of segregation occurs naturally in corn from the southwestern United States and Latin America (Rhoades, 1952).

In *Drosophila melanogaster,* the phenomenon known as "nonrandom disjunction" differs from the cases cited in that no specific genetic constitution is responsible for an observed abnormal pattern of segregation, but that, rather, the condition depends on the presence, in one female, of structurally different, or heteromorphic, homologous chromosomes (Novitski, 1951). Such a situation is named *chromosomal meiotic drive.* In *D. melanogaster* the physically shorter chromosome is included in the functional egg nucleus more frequently than the longer. All the chromosomes of the complement appear to be subject to this effect. It has been demonstrated in females heterozygous for chromosomal aberrations such as deficiencies (Novitski, 1951), translocations (Zimmering, 1955), and in females carrying attached-X chromosomes (Weltman, 1954), and other compound-X chromosomes (Novitski and Sandler, 1956).

In the mouse, as many as 95 per cent of the functional sperm from a male heterozygous for certain tailless alleles ("t-alleles") may carry those alleles whereas only 5 per cent have the normal alleles (Dunn and Gluecksohn-Waelsch, 1953; Dunn, 1953). Some of these alleles are known to be lethal in the homozygous condition and therefore should be held in check by selection, according to classic population genetic theory. It has been shown, however, that the tailless alleles are widespread in natural populations in the United States (Dunn, 1955; Dunn and Suckling, 1956) and Japan (Tutikawa, 1955), reaching a high frequency (about 25 to 50 per cent) in those populations in which they occur. This includes all but one of the populations that have been adequately studied. Since nothing is known about the cytology of the meiotic divisions of the mouse with respect to the "abnormal segregation ratios" in tailless heterozygotes, it cannot be stated with certainty that meiotic drive is the responsible agent.

In the examples cited, the recovery of the preferred chromosome (or allele) ranges from about 60 per cent in corn and *D. melanogaster,* to almost 100 per cent in the mouse and *D. pseudoobscura.* These values may not be representative in that less deviating cases would be difficult to detect, even in experimentally well-known forms. It is possible that less striking

cases of drive are more common, and to be found in a wider array of species, than these examples suggest.

POSSIBLE EFFECTS OF MEIOTIC DRIVE ON HUMAN POPULATIONS

Meiotic drive can cause a chromosome or chromosome segment to increase in frequency (or even to become fixed) in a population even if the chromosome or chromosome segment is inseparably associated with alleles imparting a reduced fitness to the organism carrying them. The fate, in a population, of a chromosome increased in frequency by meiotic drive yet decreased in frequency by negative selection depends, of course, on the relative magnitudes of the two effects. A mathematical model for essentially this situation has been presented by Prout (Appendix to Dunn, 1953). From such a model, the precise requirements for the incorporation into a population of a chromosome subject both to drive and to negative selection are readily derived. Without going into detail here, it can be shown that if a heterozygote produces gametes of two kinds with frequencies k and $(1 - k)$ respectively, where k is the frequency of the favored type, and if such a heterozygote has a reproductive fitness equal to f_1, then the chromosome subject to meiotic drive will be incorporated into the population provided that $2f_1k > 1$. Fixation of the chromosome may also occur, and theroetically will when $f_2 > 2f_1(1 - k)$, where f_2 is the reproductive fitness of individuals homozygous for the chromosome subject to meiotic drive.

The significance of this type of phenomenon with respect to problems of evolution has been recognized by Dunn and has been discussed by him in some detail previously (Dunn, 1953). He has also suggested that certain diseases of man (thalassemia and sicklemia), caused by dominant genes, may have reached a high frequency in the population by virtue of some type of abnormal segregation. Other possible explanations of these high frequencies are (1) high mutation rates and (2) high reproductive fitness of heterozygotes ("balanced polymorphism"). These two ideas have been thoroughly reviewed by Neel (1956), who points out that whereas either hypothesis is certainly possible, neither is completely satisfactory. The distinction between these and meiotic drive depends on an exact determination of the frequency of the dominant alleles in children of parents one of whom is heterozygous for the dominant. If meiotic drive is the effective agent, the heterozygous offspring should exceed 50 per cent of the total. The tabulations of pedigrees available at the present time do not reveal any deviations from 1 : 1. Because of the possibility of errors in classification and the high numbers required, however, the final decision awaits the accumulation of more accurate data.

For the moment let us entertain the notion that thalassemia and sicklemia are associated with chromosomal aberrations that are subject to meiotic drive. A justification for this conjecture is that *D. melanogaster* provides an excellent model. The algebraic expressions given previously indicate that meiotic drive coming about as the consequence of a chromosomal aberration, as, for instance, a translocation, which regularly produces an ap-

preciable frequency of aneuploid gamete types, could be effective only in forms in which the regular production of aneuploid zygotes (or aneuploid gametes) does not reduce the reproductive fitness of individuals carrying the aberration to such an extent that the inequality $2f_1k > 1$ does not hold. This restriction limits the situations in which meiotic drive caused by a chromosomal rearrangement could be effective. One organism in which it might very well be effective, however, is man. Suppose, for example, that a translocation arose in a human population. Roughly 50 per cent of the zygotes produced from matings involving persons carrying the translocation would be lethal, but this lethality would presumably occur very early in gestation and therefore probably cause no great decrease in the total number of offspring produced during the reproductive lifetime of the persons, since the number of children in a given family is most likely not greatly dependent on the frequency of very early abortions.

The following test might be applicable if the high frequencies of the genes for thalassemia and sicklemia are caused by meiotic drive associated with a chromosomal rearrangement of a type that produces an appreciable frequency of aneuploid types. In such a case, since a high proportion of the zygotes produced by matings involving affected individuals would be lethal, the observed frequency of dizygotic twins would be greatly reduced, whereas the frequency of monozygotic twins (such twins coming from one viable zygote) should remain unchanged. In fact, for any trait in man, a reduced frequency of dizygotic twins in the progenies of affected persons, without a concomitant decrease in the frequency of monozygotic twins, would be indicative of a departure from the ordinary rules of inheritance, and would require further interpretation. The presence of chromosomal rearrangements is one such interpretation. This sort of test has its parallel in the well-known litter-size determinations in other organisms.

One final point must be made here. Chromosomal meiotic drive in general requires for its operation the structural alteration of a chromosome. Such structural alterations arise only very rarely in experimental populations and presumably also in natural populations, including man. It is well known, however, that the frequency of such alterations can undergo tremendous increases after acute exposure of mature gametes to ionizing radiations. It would seem therefore that, in evaluating the effects on man of acute exposures to irradiation, the possibility must be considered that deleterious phenotypes associated with some chromosomal aberrations might not be selected out of the population, as is usually thought, but might increase in frequency and reach some high equilibrium value, possibly approaching fixation. For this reason, irrespective of the adequacy of the hypothesis of meiotic drive as an explanation for the cases mentioned, it is of some importance to determine whether chromosomal meiotic drive can occur in man.

EVOLUTIONARY IMPLICATIONS

It is possible that in the course of evolution certain deleterious genes have inexorably increased in frequency owing to meiotic drive, in spite of

negative selection, to the decided detriment of the species. There are several ways, however, by which natural selection might contravene the ill effects of drive. Mutation of the deleterious genes or the accumulation of modifiers or modifying systems of genes could, of course, act to remove such ill effects. In natural populations of *D. affinis*, for instance (Novitski, 1947), a recessive gene is known that causes the "sex ratio" X chromosome to reverse its action and cause the production of mostly Y-bearing sperm. It could have been selected for originally as a counteraction to the meiotic drive of the sex-ratio X chromosome. In such a general situation, the most adapted subpopulations of a species might be those in which the chromosome subject to meiotic drive has itself accumulated deleterious genes with the result that, although the chromosome is increased in frequency by meiotic drive, it does not become fixed in the population, owing to its own unfitness. There is reason to believe that the sex-ratio X chromosome in *D. pseudoobscura* is held down in the population in this fashion (Wallace, 1948). If meiotic drive, in a form, were to depend on crossing over, as it does in *D. melanogaster* females, then factors tending to reduce exchange, as for example inversions, would be selected for.

In addition to these, another hypothetical escape from meiotic drive presents itself. If the adaptive value of a species is being lowered by genes subject to meiotic drive, then there may be some immediate positive selective value to factors that drastically modify the nature of meiosis, with the result that the specific events on which the meiotic drive depends are eliminated or changed in some way. Although it cannot be proved that any one of the naturally occuring aberrant meioses now known arose for this reason, it may be suggested that occasionally the mechanisms of the meiotic divisions, ordinarily an arbitrary and conservative force in regulating the gene content of a species, become disadvantageous to the species. The result would be that genes modifying meiosis are selected for and become incorporated into the species.

The phenomenon of meiotic drive may provide a valuable tool for the study of experimental evolution, for the genetic content of a natural population could theoretically be changed by the introduction of individuals carrying chromosomes subject to meiotic drive. This suggestion may be tested with natural populations of Drosophila, or any organism sufficiently well understood genetically for chromosomes showing drive to be produced experimentally. Not only would such an experiment reveal the extent to which meiotic drive could be an effective evolutionary agent, but it would also serve as a test of certain fundamental concepts that are now considered to be the basis of evolutionary dynamics.

SUMMARY

Instances are known both from studies of natural populations and laboratory experiments in which heterozygotes produce two kinds of gametes, not with the customary equality, but with unequal frequencies. Such mei-

otic behavior will profoundly affect gene frequencies in populations, and is referred to as meiotic drive. Some of the consequences of meiotic drive on the genetic structure of natural populations, including those of man, and its evolutionary implications are considered.

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THE RELATION OF GENOME NUMBER TO RADIOSENSITIVITY IN HABROBRACON*

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Studies designed to determine the relation of genome number to radiosensitivity have been carried out on diploid and polyploid cereals where growth and survival of plants x-rayed as seeds have been measured by Fröier, Gelin, and Gustafsson (1941), Müntzing (1941), and Smith (1946), on haploid and diploid yeasts by Latarjet and Ephrussi (1949) and by Tobias (1952), on the development of diploid and triploid *Drosophila* x-rayed as embryos by Lamy and Muller (1939), and on haploid and diploid *Habrobracon* x-rayed at various stages of development by Whiting and Bostian (1931), Clark and Mitchell (1951, 1952). Although in most of these studies a greater resistance with higher ploidy has been demonstrated, in other studies no change in radiosensitivity with ploidy has been found (Lamy and Muller, 1939; Clark and Mitchell, 1951) and in still other cases an increase in radiosensitivity associated with higher ploidy has been reported (Clark and Mitchell, 1952; Mortimer, 1952.

Within the species *Habrobracon juglandis* (Ashmead) haploids and diploids occur normally and can be obtained in the same cultures and studied at identical stages of development. The radiosensitivity varies considerably during the life cycle from lethal doses that are less than 100 r for embryos in the cleavage stages to more than 100,000 r for adults. The wide range of tolerance to x-radiation and the many different developmental and metabolic conditions during the life cycle indicated that a comparison of haploid and diploid radiosensitivity at different stages of development in this organism would be important in the evaluation of the relationship between genome number and radiosensitivity. The investigations on this subject are considered in the present paper.

EXPERIMENTAL METHODS

Females from stock 33 were crossed to males from stock 1 and the progeny, consisting of haploid males and diploid females, were x-rayed at known stages of development. In previous work by Clark and Kelly (1950) and Clark and Mitchell (1951), comparisons of diploid males, haploid males and diploid females have shown that the differences in radiosensitivity are due to ploidy rather than to sex. Since diploid males are more difficult to obtain in large numbers they were not used in the present studies. The

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females were used as the representative diploids to be compared with the haploid males.

This comparison of haploid and diploid radiosensitivity in Habrobracon has been in progress for a number of years. The experiments reported here however were carried out at the Brookhaven National Laboratory during 1953. The x-rays were generated with a G. E. Maxitron facility operated at 250 KVP and 30 ma. The x-rays were filtered through 1 mm of aluminum and delivered at 600 r/minute or at 2200 r/minute. The HVL was equal to 0.2 mm copper.

RESULTS

Embryos between 24 and 26 hours of age were irradiated and the number of individuals that survived to at least the pupal stage, the per cent eclosion, and the incidence of female progeny were recorded (table 1). At 30°C, hatching occurs 30 hours after oviposition. It is shown (1) that the per cent eclosion from cocoons is the same for both males and females, (2) that the incidence of female progeny does not change even though there is a marked and an orderly decrease in the number of male and female adults with increasing dose. These data show that haploid males and diploid females are equally radiosensitive when treated at this stage of development.

Cultures containing haploid males and diploid females were irradiated at known postembryonic stages. Within the range of doses used an arrest of

TABLE 1

ECLOSION AND SEX RATIOS FROM X-RAYED EMBRYOS
(Age when x-rayed, 24–26 hours)

Dose (Minutes) at 600 r/minute	Males			Females			Incidence of Females	
	No. Pupae	No. Adults	Per cent Eclosion	No. Pupae	No. Adults	Per cent Eclosion	Pupae	Adults
0	149	128	86 ± 3	178	165	93 ± 2	.54 ± .03	.56 ± .03
2½	45	41	91 ± 4	58	50	86 ± 5	.56 ± .05	.55 ± .05
3	159	105	66 ± 4	238	174	73 ± 3	.60 ± .02	.62 ± .03
3½	76	16	21 ± 5	136	31	23 ± 4	.64 ± .03	.66 ± .07
4	40	1	2 ± 2	70	0	0	.64 ± .05	....

TABLE 2

INCIDENCE OF COCOONS AND ADULTS FROM X-RAYED LARVAE
(age when x-rayed, 38–40 hours)

Dose (minutes) at 600 r/min.	No. Larvae	No. Cocoons	No. Adults	Cocoon / Larva	Adult / Cocoon
0	322	310	295	.96	.95
2	90	87	71	.97	.82
2½	286	278	214	.97	.77
3	241	218	165	.90	.76
3½	211	177*	107	.84*	.60
4	243	227	56	.93	.25

*This value is low because some cocoons were discarded due to error in handling.

development was noted for some individuals and occurred at the pigmented pupal stage. A typical example of this is presented for the youngest larvae that were irradiated (table 2). Of 322 untreated larvae, 310 spun cocoons, and 295 emerged as adults. Of 243 larvae exposed to the highest dose of x-rays, 227 spun cocoons, while only 56 eclosed as adults. Thus, there appears to be no difference between the incidence of pupae obtained from the untreated and treated larvae. The recognizable effects appear after pupation and are revealed in the adult/cocoon ratios. This is most fortunate in the present experiments since the irradiated larvae have developed to a stage where their sex can easily be determined. The quantitative measure

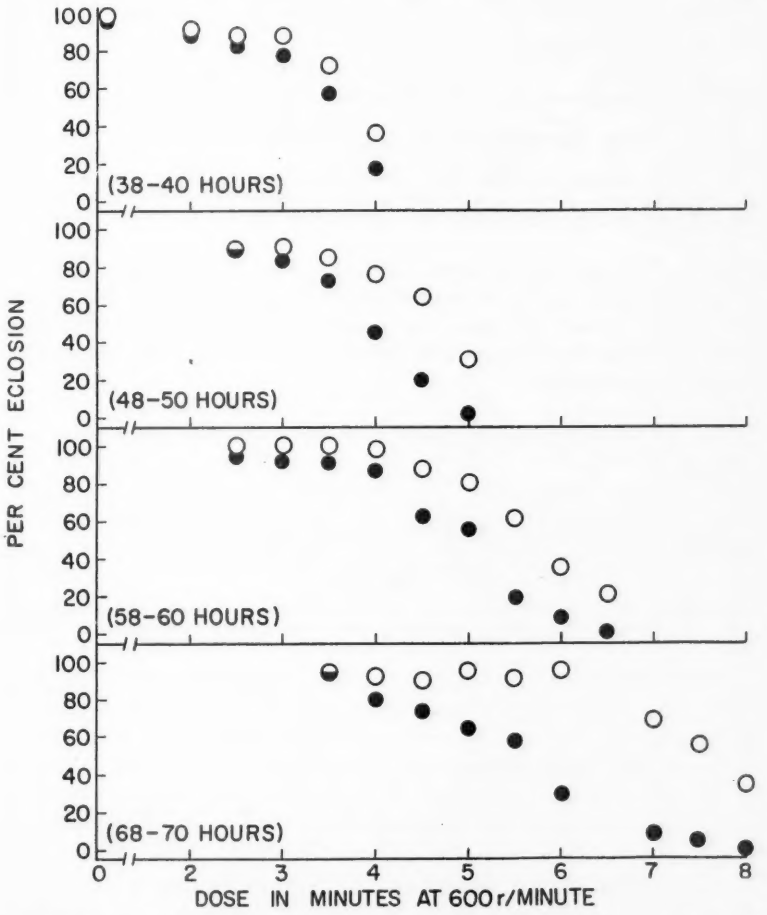

FIGURE 1. Dosage-eclosion curves for haploids and diploids x-rayed as 38-40, 48-50, 58-60, 68-70 hour old larvae. Open circles indicate per cent eclosion for diploid females; dots indicate per cent eclosion for haploid males.

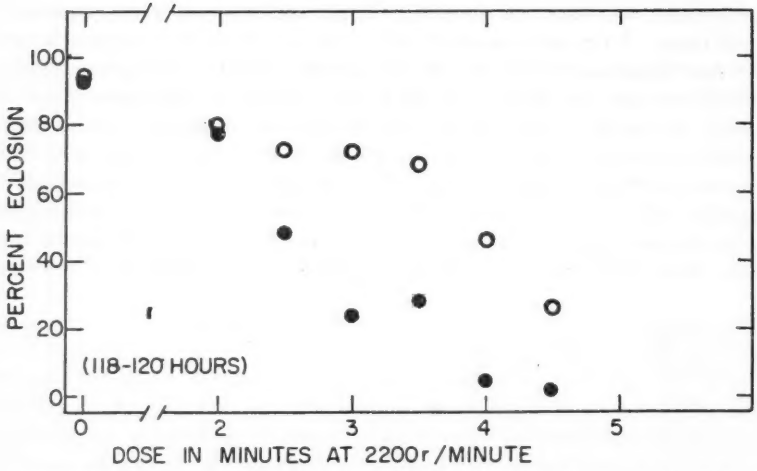

FIGURE 2. Dosage-eclosion curves for haploids and diploids x-rayed as 118–120 hour old prepupae. Open circles indicate per cent eclosion for diploid females; dots indicate per cent eclosion for haploid males.

of survival that was used in these experiments, the eclosion ratio, is defined as the ratio of the number of haploid males or diploid females that emerged from cocoons to the total number of individuals whose sex could be determined. Cocoons of uneclosed individuals were opened in order to determine the sex of the occupant.

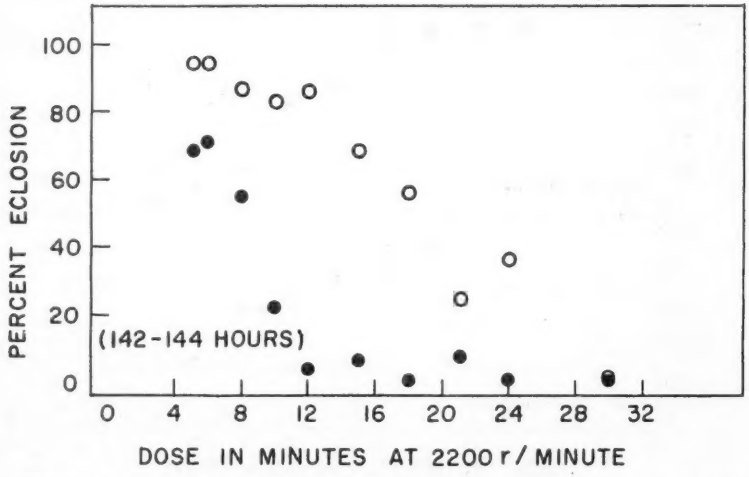

FIGURE 3. Dosage-eclosion curves for haploids and diploids x-rayed as 142-144 hour old pupae. Open circles indicate per cent eclosion for diploid females; dots indicate per cent eclosion for haploid males.

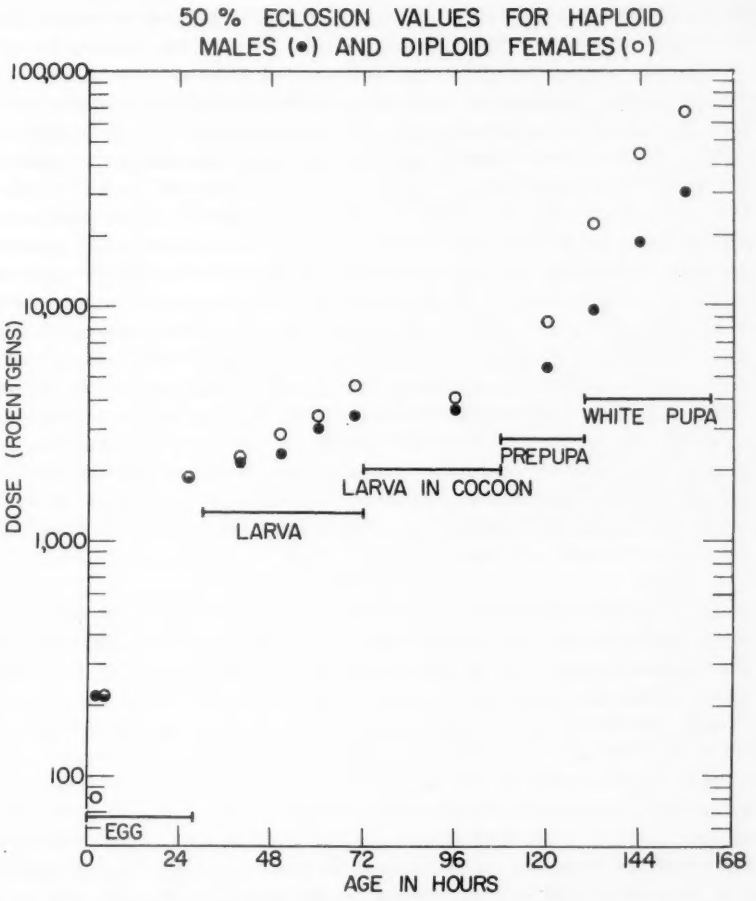

FIGURE 4. The dose in roentgens required to give 50 per cent eclosion at various stages of development. Open circles—diploid females; dots—haploid males.

Survival curves for haploid males and diploid females of different ages at the time of exposure were obtained and some of these are shown here (Figures 1, 2, 3). Comparison of per cent eclosion between haploids and diploids of the same age group shows that the differential radiosensitivity is not constant throughout development. The haploid and diploid larvae are more nearly alike in their resistance to x-rays than are haploid and diploid pupae. These differences in radiosensitivity are revealed by the figures showing per cent eclosion for larvae of four different ages (figure 1), for a prepupal stage (figure 2) and for a pupal stage (figure 3).

From these eclosion curves an x-ray dose that permits 50 per cent eclosion was estimated. Comparison of the radiosensitivity of haploids and

diploids of the same age is made in terms of this 50 per cent eclosion (survival) value (figure 4). The estimation of the 50 per cent survival for the two earliest stages is based on previously published data (Clark and Mitchell-1952). Examination of relative radiosensitivities throughout the life cycle shown in figure 4 reveals the following facts. (1) During the cleavage stages of the embryo, haploids are more resistant than diploids. (2) At later embryonic stages, haploids and diploids are equal in radiosensitivity. (3) Young diploid larvae are only slightly more resistant than haploids while old diploid larvae are markedly more resistant than comparable haploids. (4) During the stage when the larvae are in cocoons there is a decrease in the resistance of diploids but not haploids to x-rays; there is, accordingly, a decrease in their differential radiosensitivity at this stage. (5) This difference in radiosensitivity between haploids and diploids is greater at the prepupal stage than at the larval stage and increases still more at the pupal stage. (6) During most of the pupal stage (figure 4 and Clark and Mitchell, 1951) the resistance to x-rays of haploids and diploids increases exponentially and the differential radiosensitivity is constant. These data show that there is no simple correlation between genome number and radiosensitivity throughout the life cycle of Habrobracon.

DISCUSSION

Injury to a germ cell may be passed on to all of the descendent cells of a developing organism and the presence of a nuclear-type injury may be established by studies of lethal mutations. The x-radiation of an entire organism however results in damage to some of the cells only.

Under these conditions regulation and increased activity on the part of uninjured cells may occur and this makes more difficult the determination of the site and extent of the initial injury. There is evidence that the nucleus of somatic cells is damaged by x-radiation. Much of this direct evidence comes from studies of chromosomal aberrations in x-rayed somatic cells (Fröier, Gelin, and Gustafsson, 1941; Smith, 1946) but a great amount of such evidence is indirect, being an extrapolation from the enormous amount of work done on the germ cells (Muller, 1950). The present type of study in Habrobracon seems to allow the opportunity to study this problem in a complete organism and for somatic tissue.

The fact that differences in radiosensitivity between haploids and diploids are obtained suggests that the nucleus is involved and is the primary site of injury from irradiation. The lack of a constant haploid-diploid sensitivity during development may indicate that secondary factors may modify the response of the organism to this initial injury. When one considers the thousand-fold range in sensitivity and the complex metabolic changes during development it is perhaps not surprising to find an absence of constant differential sensitivity between haploids and diploids. Since the problem is defined in terms of haploids and diploids, one must consider the extent to which a genetic interpretation can explain these data.

During the cleavage stages of development diploids are more radiosensitive than haploids. Diploid nuclei with twice as many chromosomes as haploid nuclei would furnish two times the target and have twice as many aberrations. This has been shown for plant material where the number of aberrations per tetraploid cell has been shown to be twice that for the diploid cell (Smith, 1946, Bishop, 1952) and the ratio of breaks per chromosome remains constant. This phenomenon of greater sensitivity with increased ploidy can be explained by assuming the induction of dominant lethals. Whiting (1945) and Heidenthal (1945) have shown that dominant lethals are quite frequent in Habrobracon. Mortimer (1952) has obtained a greater sensitivity to x-rays for tetraploids than for diploids in yeast and has proposed that dominant lethal mutations have been induced. Whiting (1945) has shown that dominant lethals induced in unlaid Habrobracon eggs result in breakage-fusion-bridge phenomena during cleavage. Muller (1954) has pointed out that chromosome breaks may lead to an interference with normal chromosomal allocation during mitosis and that a bridge by itself, regardless of the number of chromosomes or genomes of the cell can lead to cell death. The present work can add to this only that a single bridge in a diploid is as deleterious to the cell as a single bridge in a haploid and that there is twice as much chance for chromosome breakage and bridge formation to occur in the diploid nucleus.

During the larval and pupal stages of development, haploids are more radiosensitive than diploids. This is comparable to the situation that has been found for haploid and diploid yeasts and for diploid and tetraploid cereals where increased resistance can be correlated with an increase in ploidy. In all of the above cases the explanation of lethality has been based upon the concept of recessive lethal mutations. In diploids there is less chance for homozygous deficiencies or inactivation of genes to occur. A genic loss in a haploid is irreparable while such a loss in the diploid could be compensated for by the unaffected allele on the homologous chromosome. Since gene action and influence is important during these periods of rapid differentiation the loss of a particular kind of gene would lead to cell death. Despite the increased initial injury to diploids there would be less genetic imbalance than in haploids. Thus, lethality during these stages would be due primarily to recessive lethals.

If one assumes that dominant lethals are more influential during some stages and that recessive lethals are more effective during other stages, then the relative radiosensitivities of haploids and diploids at any particular stage can be explained in terms of a balance between the dominant and recessive lethals. If this interpretation is valid then it can be seen that death due to dominant lethals appears early in development and to recessive lethals appears later in development. Younger individuals are more sensitive because they are more susceptible to slight chromosome and gene changes than are the older ones. Diploids are then more sensitive because they have received twice the injury that haploids have received and the

young organisms are unable to compensate for these changes. Under these conditions it would be possible that a particular type of aberration would be scored as a dominant lethal at one stage of development but would be viable if incurred at a later stage of development.

Young larvae when irradiated continue to develop until the pigmented pupal stage (table 2) and the arrestment of development, if it occurs, takes place at this stage. Also, when embryos are irradiated during cleavage, development is arrested during cleavage (Clark and Mitchell-1952). The fact that there appear to be specific stages at which development is arrested seems to indicate that a more generalized type of nuclear effect may occur. Nuclear effects not necessarily dealing with chromosome breaks are possible since a chromosomal effect involving centromeres has been proposed by Marshak and Bradley (1944) who found that the x-ray induced inhibition of mitosis in cereals is inversely proportional to the number of sets of chromosomes but independent of chromosome length. Fröier, Gelin and Gustafsson (1941) have proposed from their work on polyploid cereals that an extrachromosomal nuclear effect may occur in radiation damage. Studies relating radiation sensitivity to DNA synthesis have been reported (Sparrow, 1952, Hevesy, 1952). It is possible in Habrobracon that some such nuclear controlled synthesis affecting perhaps nucleic acids or proteins may be involved and that interference with this synthesis prevents development to the adult stage. During the embryonic and early larval stages of development where haploids and diploids are approximately equal in radiosensitivity the nuclear controlled syntheses are presumed to be of the same magnitude. The varying differential radiosensitivity found at different stages of development is presumed to be paralleled by a differential synthesis between haploids and diploids.

SUMMARY

Haploids and diploids of *Habrobracon* are compared with regard to their sensitivity to damage by x-rays. The relative radiosensitivities were different for different stages of development. The bearing of these data on determining the site of injury from x-radiation is discussed.

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ISOAGGLUTINATION IN FISH

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Attention has been called in earlier papers (cited below) to the potential value of erythrocyte antigens as aids in distinguishing separate breeding populations within single species of fish, and in studying the migrations and evolutions of these populations. Investigation of this possibility has disclosed individual variations in the antibody content of the sera of two species of tuna (Cushing 1953a,b), species specific differences among fish erythrocyte antigens (Cushing and Sprague 1952, 1953), and individual variations in the erythrocyte antigens of the oceanic skipjack (Cushing, in press). The present paper describes an isoagglutinin system that has been found in a population of brown bullheads (*Ictalurus n. nebulosus* Le Sueur). This system has particular interest from an evolutionary point of view for it extends our knowledge of the occurrence of reciprocal isoagglutination to the class Pisces. In addition it raises the possibility that natural isoantibodies may prove useful in studying antigenic variations within other species than those reported here.

MATERIALS AND METHODS

The bulk of the data to be presented was obtained from samples of a population of brown bullheads thriving in Laguna Blanca, Hope Ranch, Santa Barbara, California. The population was established about eight years before this study was made, from a planting of some dozen fish taken from Zaca Lake, Santa Barbara County. At the present time the population is numerous, to judge by the ease with which individuals were caught. These were taken with hook and line, averaged ten inches in size, and were kept in tanks until used. All individuals were very heavily parasitized by the metacercariae of a microphallid trematode, which were identified by Dr. D. M. Wootton and Frank Hester.

Further studies were conducted on channel catfish, *Ictalurus p. punctatus* Le Sueur, purchased from the Rainbow Meadows Trout Farm, Santa Paula, Ventura County, California. Studies that were made on fishes of other families are also noted.

Bled from the tail, the brown bullheads usually yielded two to four ml. of blood; bled from the heart they could yield up to one ml. of blood per bleeding, and be bled at repeated intervals. Cells kept as long as three weeks in refrigerated clots. Immediately before use they were washed in 1 percent NaCl solution (an arbitrarily selected concentration). In the individual agglutination tests two drops of light cell suspension (2 percent) were put with two drops of serum dilution in a small test tube (10 mm. × 75 mm.).

After five minutes the tube was centrifuged at 1000 R.P.M. for thirty seconds. The degree of agglutination was measured by observing the re-suspension of cells following gentle tapping of the tube.

Readings were recorded as 4+ for complete agglutination, 0 for no agglutination, and 3+, 2+, and 1+, for intermediate degrees of agglutination. Controls were run throughout and critical tests were repeated at least once. The relationships to be described were readily observed by this technique. Unless otherwise noted, each test was made at a serum dilution of 1 in 5 at room temperature. Generally speaking, the serological reactions were not observed to be sensitive to minor deviations from the roughly standard conditions just described. Certain tests, specified below, were carried out at other dilutions, for longer periods, and at refrigeration temperatures (approximately 5°C.). The antibodies in undiluted catfish serums were not observed to be affected by repeated freezing and thawing.

VARIATION IN THE ERYTHROCYTE ANTIGENS OF *ICTALURUS N. NEBULOSUS*

Two erythrocyte antigens (designated 1 and 2) and their corresponding isoagglutinins (anti-1 and anti-2) were found to vary with individual fish in Laguna Blanca. An inverse relationship appears to obtain among the antigens and antibodies concerned, analagous to that described for the human A, B, 0 blood group system.

Evidence for these relationships is presented in table I which shows the results of cross-matching the cells and sera of 17 fish (+ shows tests where

TABLE 1

ISOAGGLUTINATION REACTIONS OF BROWN BULLHEADS
(*ICTALURUS N. NEBULOSUS* LE SUEUR)

Sera (1:5)	CELLS																	
	Antigen 1						Antigens 1,2			Antigen 2					Neither Antigen		
	62	69	80	82	88	93	67	75	87	46	63	70	81	91	66	79	83
62	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0
69	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0
80	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0
82	−	−	0	0	0	0	−	0	0	−	−	−	0	0	−	0	0
88	−	−	0	0	0	0	−	0	0	−	−	−	+	+	−	0	0
93	−	−	0	0	0	0	−	0	0	−	−	−	0	0	−	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
63	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
70	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
81	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
91	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
66	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0
79	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
83	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0

TABLE 2

ABSORPTIONS OF BROWN BULLHEAD SERA
(*ICTALURUS N. NEBULOSUS* LE SUEUR)

Sera (1:5)		CELLS									
		Antigen 1			Antigens 1,2		Antigen 2		Neither Antigen		
		62	80	93	75	87	63	91	79	83	66
Serum of 79 (anti-1)	*Antigens*	4+	3+	3+	4+	2+	0	0	0	0	0
Abs/w cells of 80	1	0	0	0	0	0	0	0	0	0	0
" " " 75	1,2	0	0	0	0	0	0	0	0	0	0
" " " 91	2	4+	3+	4+	4+	3+	0	0	0	0	0
" " " 83	0	4+	4+	4+	4+	4+	0	0	0	0	0
Serum of 62 (anti-2)		0	0	0	2+	2+	2+	2+	0	0	0
Abs/w cells of 80	1	0	0	0	2+	2+	2+	2+	0	0	0
" " " 75	1,2	0	0	0	0	0	0	0	0	0	0
" " " 91	2	0	0	0	0	0	0	0	0	0	0
" " " 83	0	0	0	0	2+	1+	2+	2+	0	0	0
Serum of 66 (anti-1,2)		3+	4+	3+	3+	3+	1+	2+	0	0	0
Abs/w cells of 80	1	0	0	0	2+	2+	1+	2+	0	0	0
" " " 75	1,2	0	0	0	0	0	0	0	0	0	0
" " " 91	2	3+	4+	3+	2+	3+	0	0	0	0	0
" " " 83	0	3+	4+	3+	3+	4+	1+	2+	0	0	0

definite agglutination occurred, 0 shows where no reaction occurred, and a dash shows tests that were not made). Each cross-matching was made at room temperature, and at 5°C, with many being made several times in addition. (Exceptions were nineteen combinations that were made at room temperature only). All the 268 cross-match combinations observed were consistent with the relations shown in table I. They were also consistent within their own repetitions excepting for a few minor deviations that could readily be explained on the basis of low titer antibody or in the case of some reactions involving two fish (Nos. 46 and 63) apparent fluctuations in antibody titer during captivity. This last point will require further study for its confirmation, but is not without precedence in its occurrence, at least among mammals (Stone, in press). While anti-1 and anti-2 have never been found associated with incompatible erythrocyte antigens, a number of fish were found whose serum did not contain detectable antibody (e.g. Nos. 82, 93 in table I). This is not inconsistent with the scheme presented, particularly since it was found that the antibody titers of different fish varied greatly between extremes of very low titers to those of 1 in 80 and 1 in 160. The antibodies produced by fish lacking both antigens 1 and 2 were found to be anti-1, excepting for fish No. 66 which also produced anti-2, but in lesser amount. The reactions of serum from this fish were consistently positive with cells of antigen 1 and antigen 1,2, but weaker and more difficult to establish with all the cells of antigen 2.

Many combinations were cross-matched other than those shown (approximately 1316 among 100 fish). The majority of these were made before the

relations shown in table I had been worked out. As it was not possible to keep fish and cells intact through all the experiments, reciprocal observations could not be obtained in many cases. However, this data is consistent with the scheme presented.

Additional evidence supporting the proposed system is given by the absorption experiments shown in table 2. These are representative of a larger group of such experiments (approximately 12 sera absorbed with cells of 50 fish). They involve fish referred to in table I and show that two distinct antibodies occur which can be removed singly or in mixtures by cells of the proper type, and which are not removed by cells of other types.

ADDITIONAL OBSERVATIONS ON THE GENUS ICTALURUS

The cells and sera of 20 individuals of the channel catfish were cross-matched in 371 of the 400 possible combinations. All tests were negative excepting that the serum of one fish (No. 13) agglutinated the cells of 13 fish, and failed to agglutinate the cells of the seven others.

Various combinations of cells and sera of the two catfish species were made. Individual sera obtained from the channel catfish differentiated cells of individual brown bullheads as shown in the results of matching the sera of eighteen channel catfish with the cells of sixteen brown bullheads. At least three specificities were found. All cells reacted with one serum, some cells failed to react with any sera, and other cells reacted with certain sera only. The antigenic basis for these last reactions appeared relatively simple, and to involve the presence or absence of antigen 1. However, the necessary absorption and cross-matching tests could not be performed, so that no final conclusions can be reached on this point. Combinations of the cells of channel catfish with the sera of twelve brown bullheads showed that three sera agglutinated all cells utilized. These observations were not extended, but they do show that further antigenic complexities exist among the two species. A few cases of isohemolysis were observed among the population of brown bullheads, but these were not studied further. Hemolysis was not observed within any combinations involving the two species of catfish.

Cells of the brown bullhead were not agglutinated by an anti-sheep cell serum at a dilution of 1 in 50 (this serum strongly agglutinated, 4 plus, both human A and sheep cells at this dilution). The cells of the brown bullhead were strongly agglutinated by human commercial typing sera that had been previously absorbed with human cells to remove the anti-A and anti-B agglutinins. The effect of absorbing these sera with catfish cells has not yet been studied.

ISOAGGLUTINATION IN OTHER SPECIES OF FISH

Isoagglutination reactions were first observed in this laboratory in July, 1954, in tests with white croakers (*Genyonemus lineatus*, Ayres) collected at Santa Barbara (Cushing and Donald Shaw, unpublished data). The study

of this population has revealed 34 positive reactions among 567 cross-matches involving 68 fish. In contrast, no positive reactions were observed among 380 cross-matches among 20 white croakers obtained approximately 100 miles south of Santa Barbara through the courtesy of Marineland of the Pacific (Palos Verdes, California).

Cross-matching of the cells and sera of ten oceanic skipjack (*Katsuwonus pelamis* Linnaeus) has shown the occurrence of isoagglutination reactions in this species, the serum of one fish weakly agglutinating the cells of four other individuals known to be carrying an antigen absent in other members of the sample (Cushing, in press).

DISCUSSION AND SUMMARY

Individuals in a catfish population varied with respect to the reactions of their erythrocyte antigens with natural antibodies found in sera of the same population. Two antigens have been found to show an essentially reciprocal relationship of occurrence with respect to the two isoantibodies that identify them. These permit the differentiation of four types of individuals: those with antigen 1, those with antigen 2, those with both these antigens and those with neither. The existence of additional antigens is shown by comparative studies involving a second species of catfish. The occurrence of isoagglutination reactions in white croakers and in oceanic skipjacks raise the probability that other isoagglutination systems will be discovered among the fishes, and suggest that this phenomenon is of widespread occurrence among the vertebrates. This suggestion is supported by the occurrence of isoagglutination reactions in some species of turtles (Bond, 1940).

The present observations are contrary to the hypothesis of Ashurst (1956) who recently has stated that "The evidence at present suggests that blood groups may have started to occur in the reptiles and so their possession is confined mainly to the warm-blooded animals i.e. birds and mammals." However, Ashurst's observations on whiting (*Gadus merlangus*), pouting (*Gadus luscus*) and plaice (*Pleuronectes platessa*) do indicate that isoagglutinin reactions either do not occur or are not to be found with equal ease in all species of fish. This indication is supported by the earlier observations of Jensen's on cod (1937), and by the results obtained with white croakers noted above.

The resemblance of the catfish isoagglutinin system to the human A, B, 0 blood groups should of course be considered in a tentative sense only, for genetic studies have not been made of this phenomenon. In addition, two brown bullhead sera reacted with certain fish among those carrying antigen 2 and others lacking both antigens. The antibodies concerned were in too low titer to study systematically, but these reactions, together with those observed in interspecific combinations, do suggest the probable occurrence of further heterogeneities and relationships similar to those described by Owen (1954) and by Stormont (1955).

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

THE SOUTHERNMOST DROSOPHILIDAE

Basden (1956) has published a study of Drosophilidae living north of the Arctic Circle. He lists 8 species of the genus Drosophila, 6 of Scaptomyza, and 9 species of other genera of the family, recorded mostly from the Arctic Finland, Norway, and Sweden. One species, *Drosophila funebris* Fabr., lives also in Greenland, and 3 species, including *D. funebris*, in Iceland. It is interesting to compare these data with what is known about the drosophilids living farthest south. In February 1956 we collected some material in the extreme southern part of South America, in the Province of Magellanes and on Tierra del Fuego, in southern Chile. Although our collecting localities lie considerably north of the Antarctic Circle, it is not likely that drosophilids will be found much farther southward, since the Southern Hemisphere has little land in the latitudes between those of Tierra del Fuego and the Antarctic continent.

Drosophila funebris was found in two fruit and vegetable stores in Punta Arenas, on the Strait of Magellan (lat. 53° 10' S.). One of these places had a fairly large and flourishing population. Six specimens were also found in a greenhouse at the Estancia Caleta Josefina, on the Bahia Inutil, Tierra del Fuego (53° 40' S.). Other localities of interest are Puerto Natales (51° 44' S.), and Balmaceda on the eastern slope of the Andes facing Patagonia (45° 53' S.). Consequently, among the approximately 650 known species of Drosophila, *D. funebris* extends its distribution farthest north as well as farthest south. It happens to be the type species of the genus, described by Fabricius in 1787; it is also one of the 8 cosmopolitan species of Drosophila, which occur, according to Patterson and Stone (1952), in all six main faunal regions of the globe. Like the other cosmopolites, it occurs mostly in close association with man, but it is apparently the most cold-adapted of the "domestic" species, and it is rare or absent in the tropics. All eight cosmopolitan Drosophilae have been found in Chile (Brncic, unpublished), but *D. funebris* is the only species known to live south of the latitude 43°-44° S. Beyond reasonable doubt, *D. funebris* has been introduced in southern Chile by human agencies.

Native drosophilids of the extreme south of Chile apparently all belong to the genus Scaptomyza. Two species, *S. melancholica* Duda and *S. denticauda* Malloch, came by the hundred to baits of fermenting banana exposed in groves of antarctic beech (Nothofagus), in two locations protected from

strong winds, near Estancia Cameron, Tierra del Fuego. Some specimens have been obtained also without bait, by sweeping with a collecting net on grassy vegetation and on flowers in Nothofagus groves and in a garden. *S. melancholica, S. denticauda,* and some individuals of *S. multispinosa* Malloch, have been collected at two points in the neighborhood of Punta Arenas, to the north of the city. It should be noted that species of Scaptomyza are common also in the Arctic (Basden 1956), although the Chilean species (Brncic 1955) are all endemics.

The apparent absence of Drosophila and its replacement by Scaptomyza in the extreme south of South America are of general biological interest. It happens that representatives of Scaptomyza occur also in temperate Europe and in North America, but seem to be rare in the tropics. Now, the Scaptomyza species of the temperate Northern Hemisphere are known to live as larvae in tunnels in leaves of living plants. The food of the adults is not well known, but they are not eager visitors of baits made with fermenting fruits, which attract Drosophila species living on fermenting plant exudates, fruits, and even on fungi. The species of Scaptomyza found in southern Chile are, on the contrary, easily attracted by fermented baits, and they are occasionally met with also in human dwellings, presumably attracted by fermenting foodstuffs. Therefore, it seems probable that Scaptomyza in southern Chile occupies both the ecological niches of leaf-mining Scaptomyza of temperate countries of the Northern Hemisphere, as well as those of other drosophilids feeding chiefly on microorganisms causing fermentation of materials rich in carbohydrates, such as fruits and tree sap.
We wish to acknowledge our obligations to the Rockefeller Foundation and to the University of Chile who provided the support for our field work, and also to Dr. Elias Sabat, Mr. J. Jones, and to Dr. Pablo Voullieme, and Mr. Norman Voullieme, all of Punta Arenas, for their kind hospitality and assistance in our work.

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